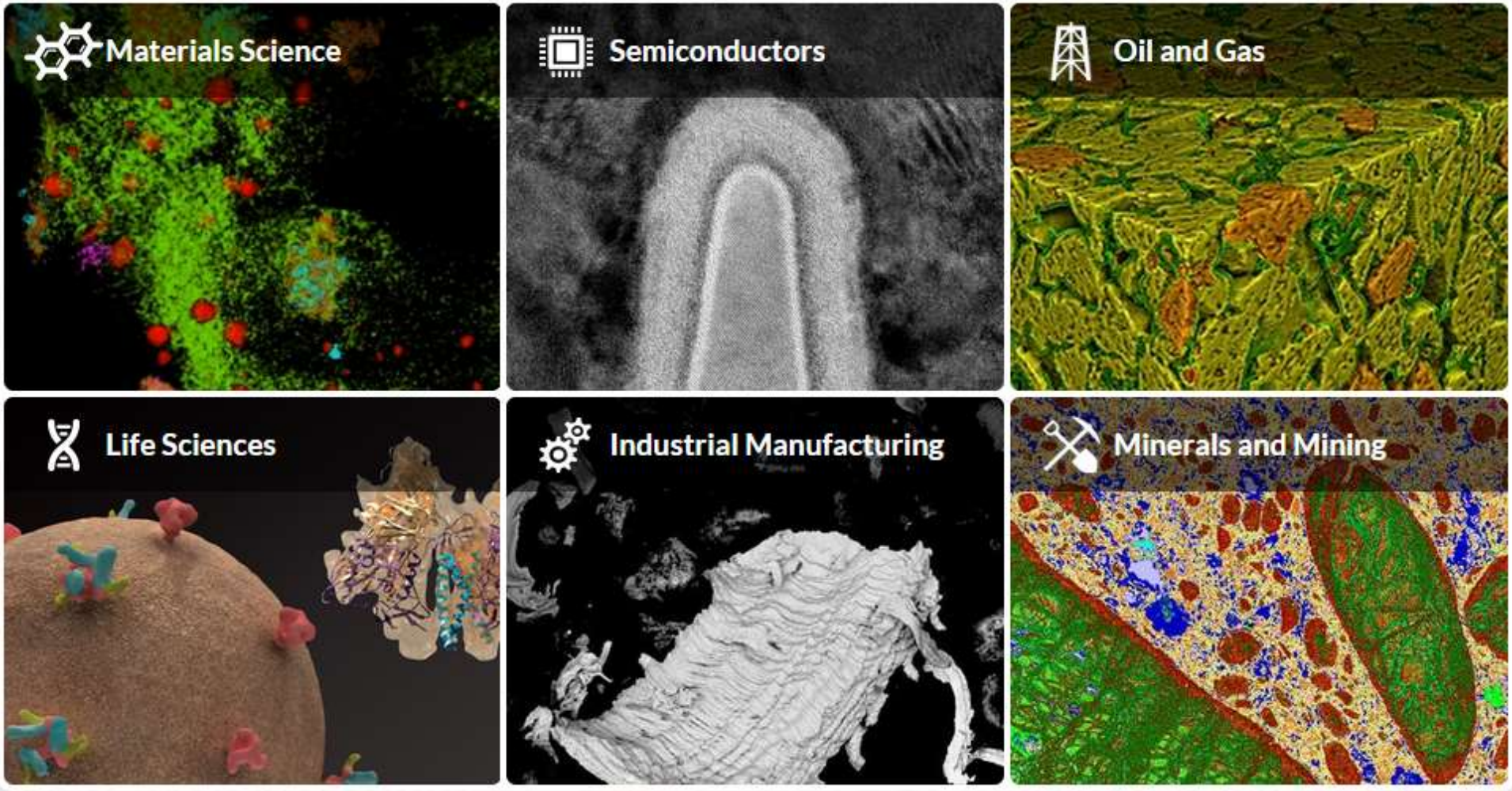
Electron microscopy workflows: from single particle analysis to volume imaging

Kristian Wadel

History

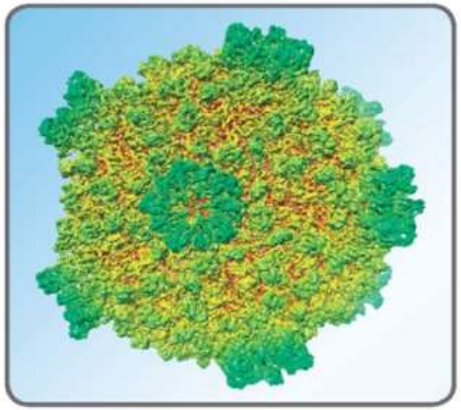
1949 – World's first production TEM introduced

FEI Company: global reach, many applications



FEI Life Science segmentation

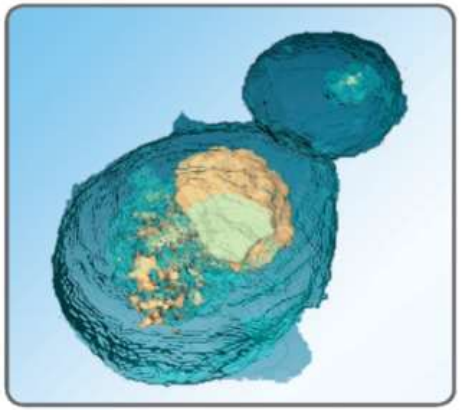
Structural Biology Solutions
Visualize life at the 3D molecular level



3.88 Å structure of Cytoplasmic Polyhedrosis virus by cryo-electron microscopy

Courtesy of Xuekui Yu, Lei Jin & Z. Hong Zhou, University of California, Los Angeles, USA

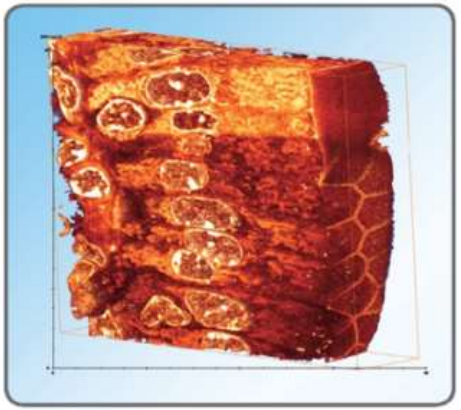
Cellular Biology Solutions
Discover life's cellular architecture in 3D



Volume rendering of the three-dimensional architecture of a dividing yeast cell

Courtesy of Sriram Subramaniam, National Institutes of Health, Bethesda, USA

Tissue Biology Solutions
Connect life's ultrastructure to the mesoscopic scale

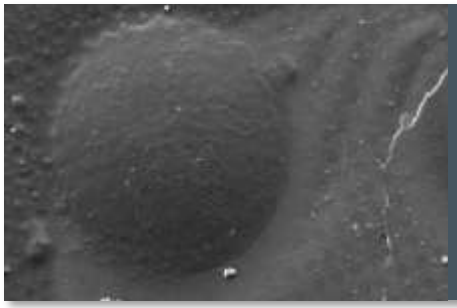


Mouse intestine epithelial tissue imaged 50 x 50 x 10 micron using a pixel size of 25 nm and section thickness of 40 nm

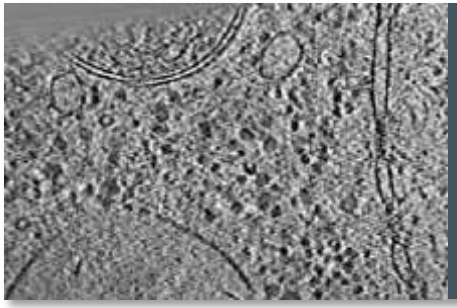
Courtesy of Paul Matsudaira, Dept of Biological Sciences, National University of Singapore

The importance of scale

Small organisms and tissues: millimeters

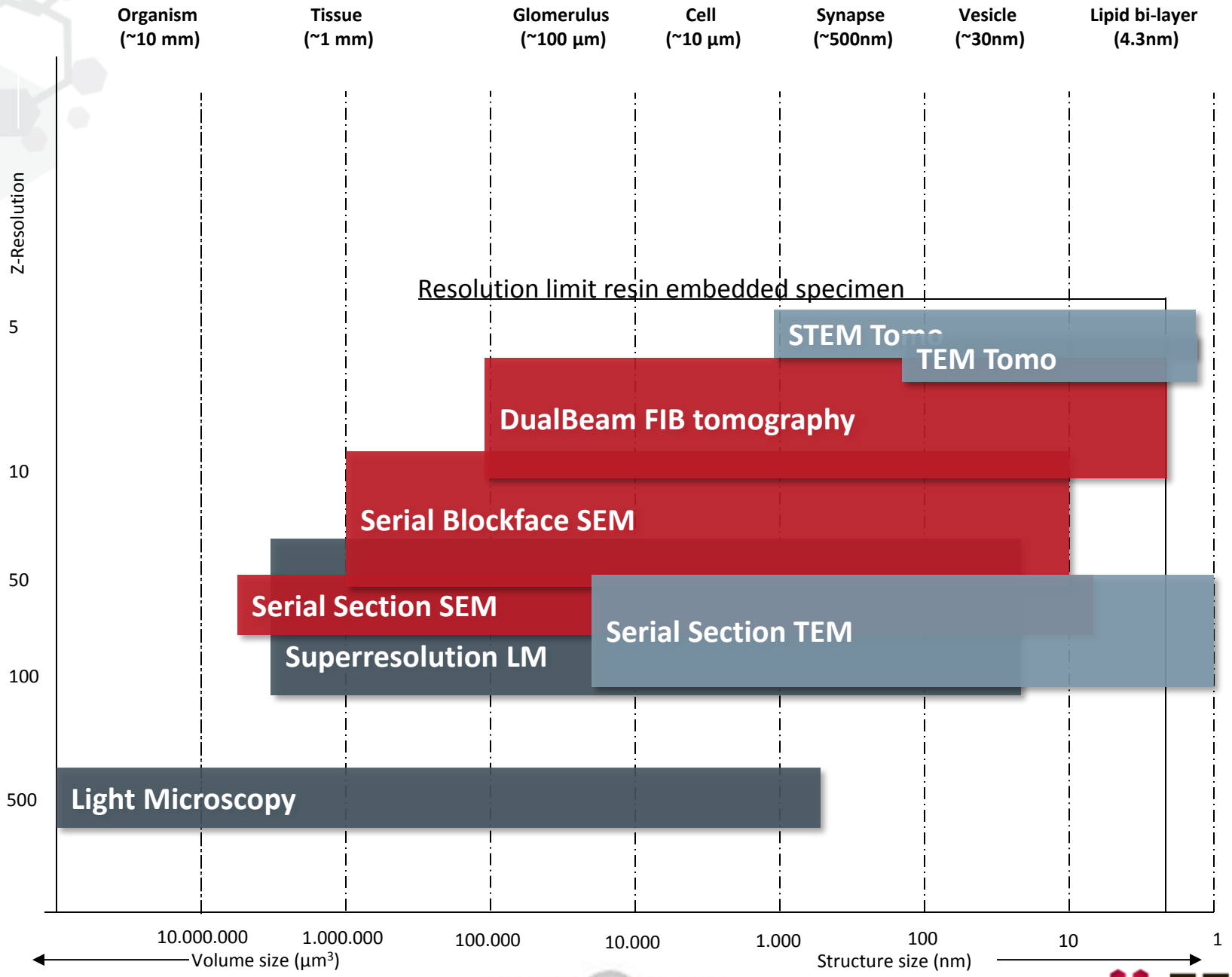


Cell: ≈300 µm^3



Tomogram: ≈0.4 µm^3

Top image: Courtesy of D. McCarthy, University College London
Middle and bottom: Courtesy of J. Mahamid, J. Plitzko and W. Baumeister, MPI for Biochemistry



Explore. Discover. Resolve.

TechnoInfo

FEI™

FEI Life Science portfolio

TEM


Tecnai


Talos


Talos Artica


Titan Halo


Titan Krios

SEM SDB


Inspect


Quanta


NovaNano


Teneo


Verios


Scios


Helios

CLEM

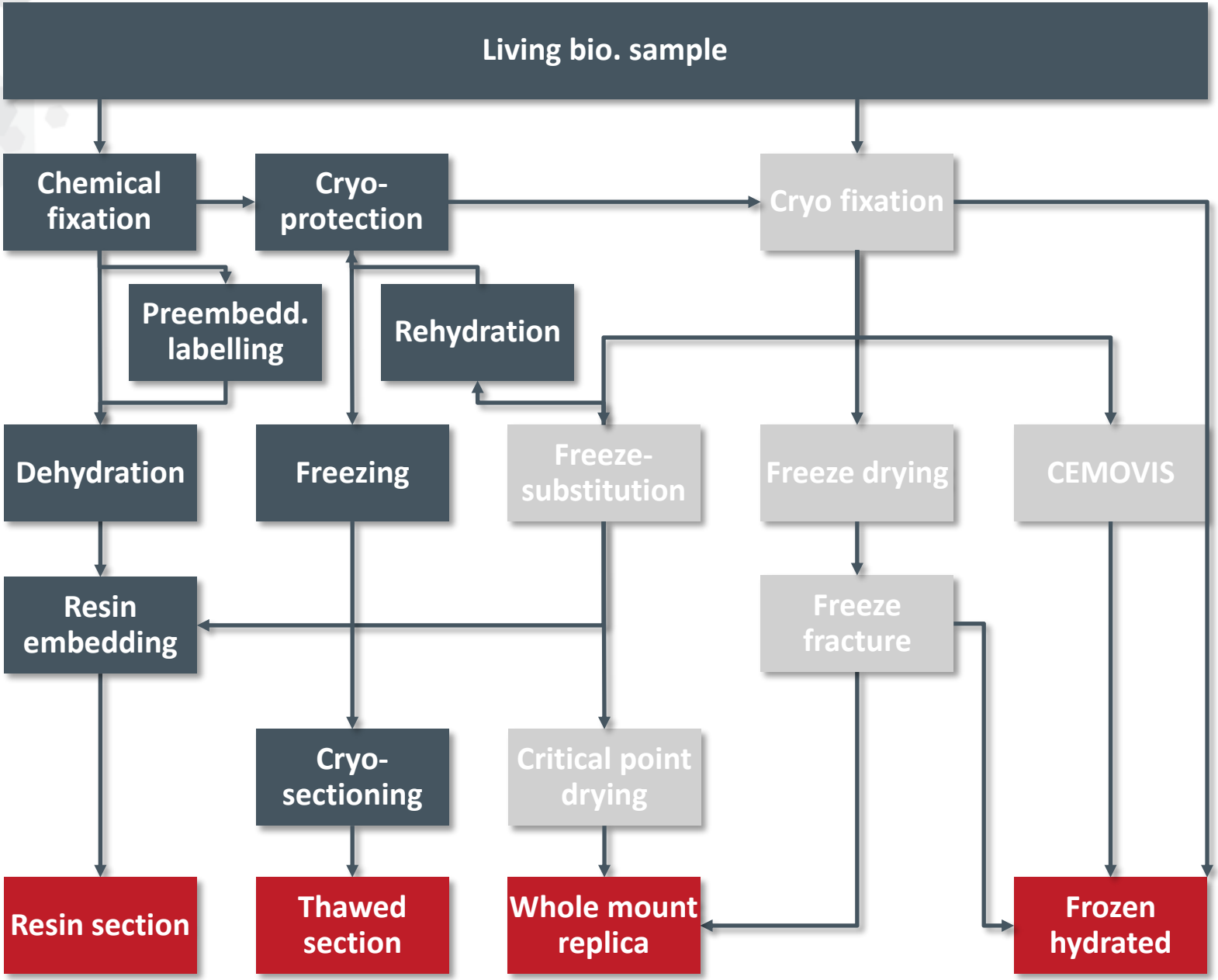

CorrSight


iCorr

Software

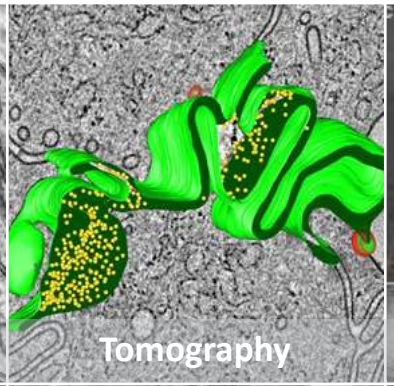
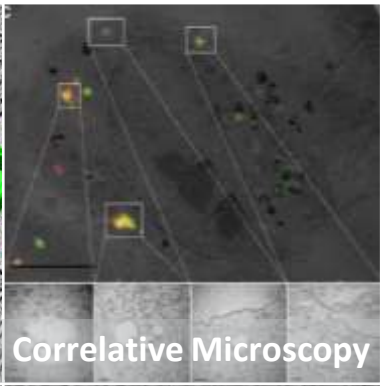
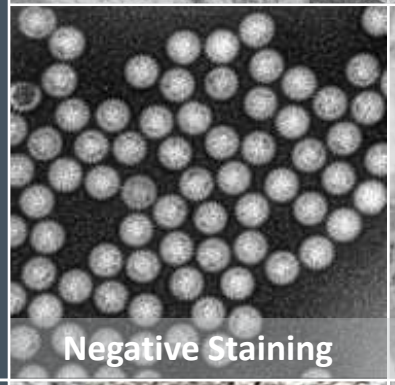
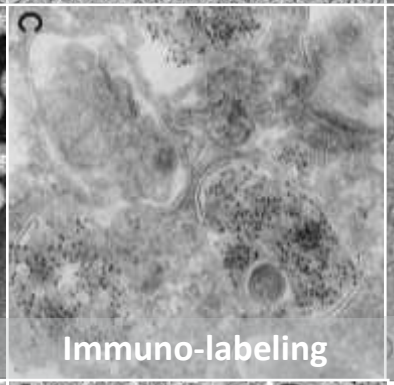
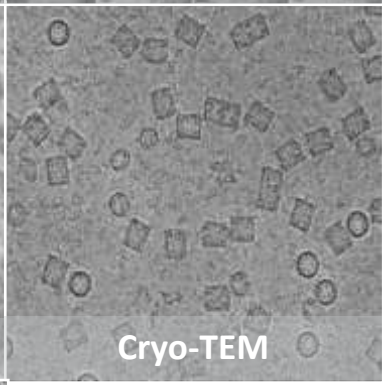
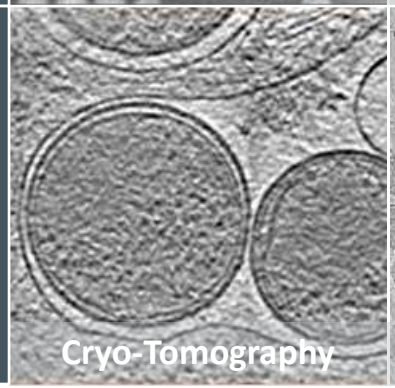
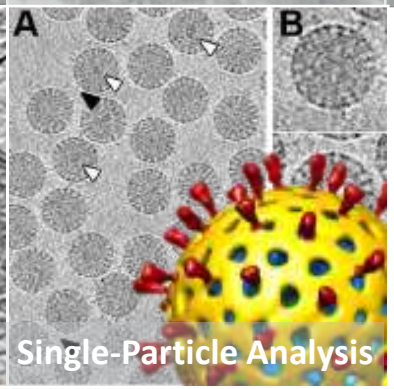
Explore. Discover. Resolve.
Maps 2

Amira® 6
for FEI Systems



Adapted from slide of Bruno Humbel, UNIL Lausanne

TEM applications

Cell & Tissue Biology	 Classical 2D	 Tomography	 Correlative Microscopy
	 Negative Staining	 Immuno-labeling	 Cryo-TEM
Structural Biology	 Cryo-Tomography	 Single-Particle Analysis	

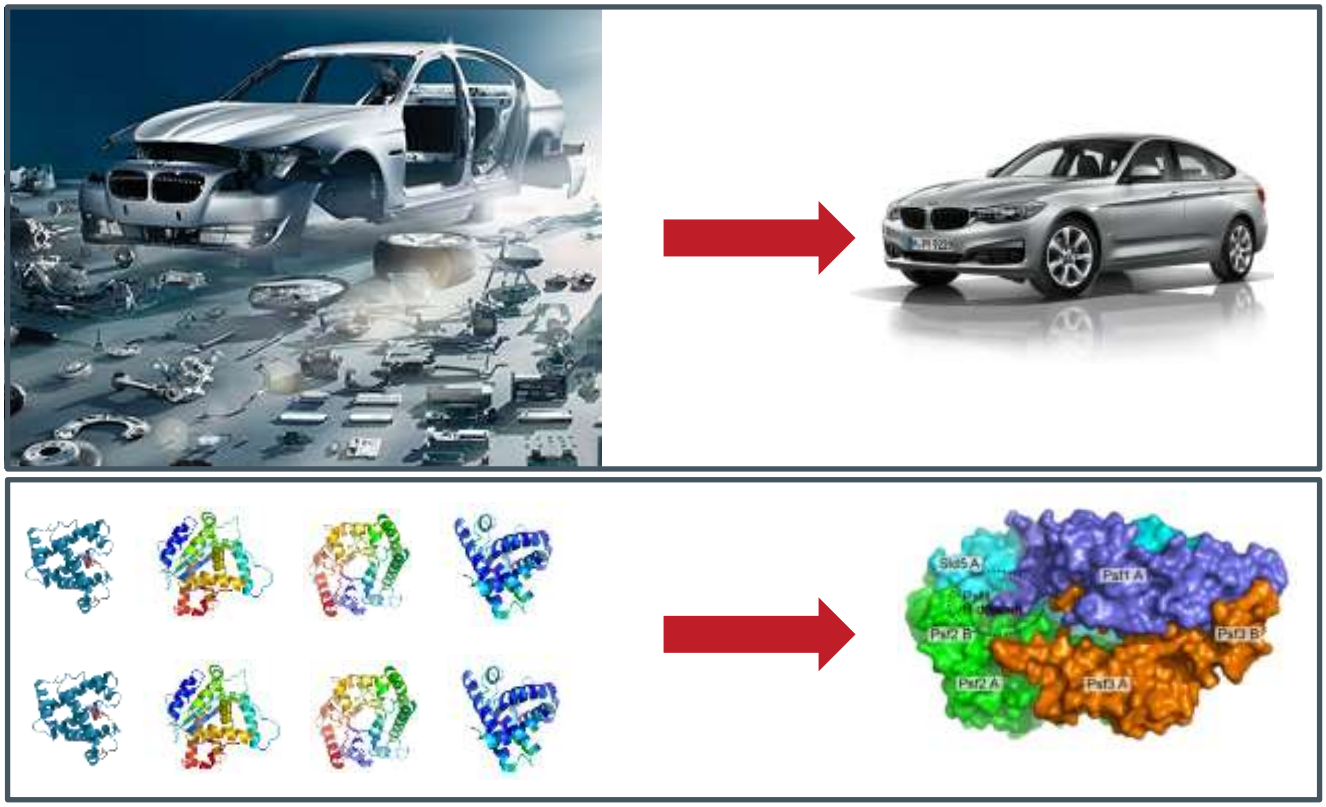
Left: Nans et al., 2014 Cell Microbiol., 16(16)
: 1457-1472

Right: Rodriguez et al., 2014 PLOS 10(5): e1004157..

Structural biology solutions

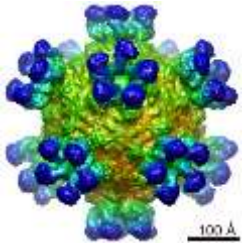
Structure-Function Relationship

Proteins act in complexes to execute their functions



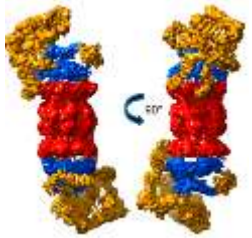
Structural Biology

- Imaging of large MDa viral complexes
  - ➢ epitope mapping, vaccine development



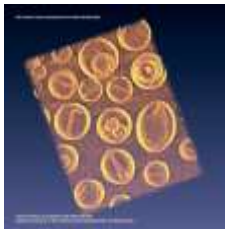
Poliovirus 135S particle and C3 Fab complex at 9.1 Angstrom resolution - EM DATA BANK (EMDB) / 5292

- Imaging of protein complexes/organelles that play crucial role in main cellular pathways
  - ➢ protein synthesis, enzymatic activities(ribosomes, proteosomes)



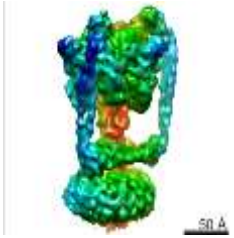
Cryo-EM map of the S. pombe 26S proteasome. Baumeister et al., 2012, PNAS 109(5): 1380-1387.

- Quality control on production of novel medications



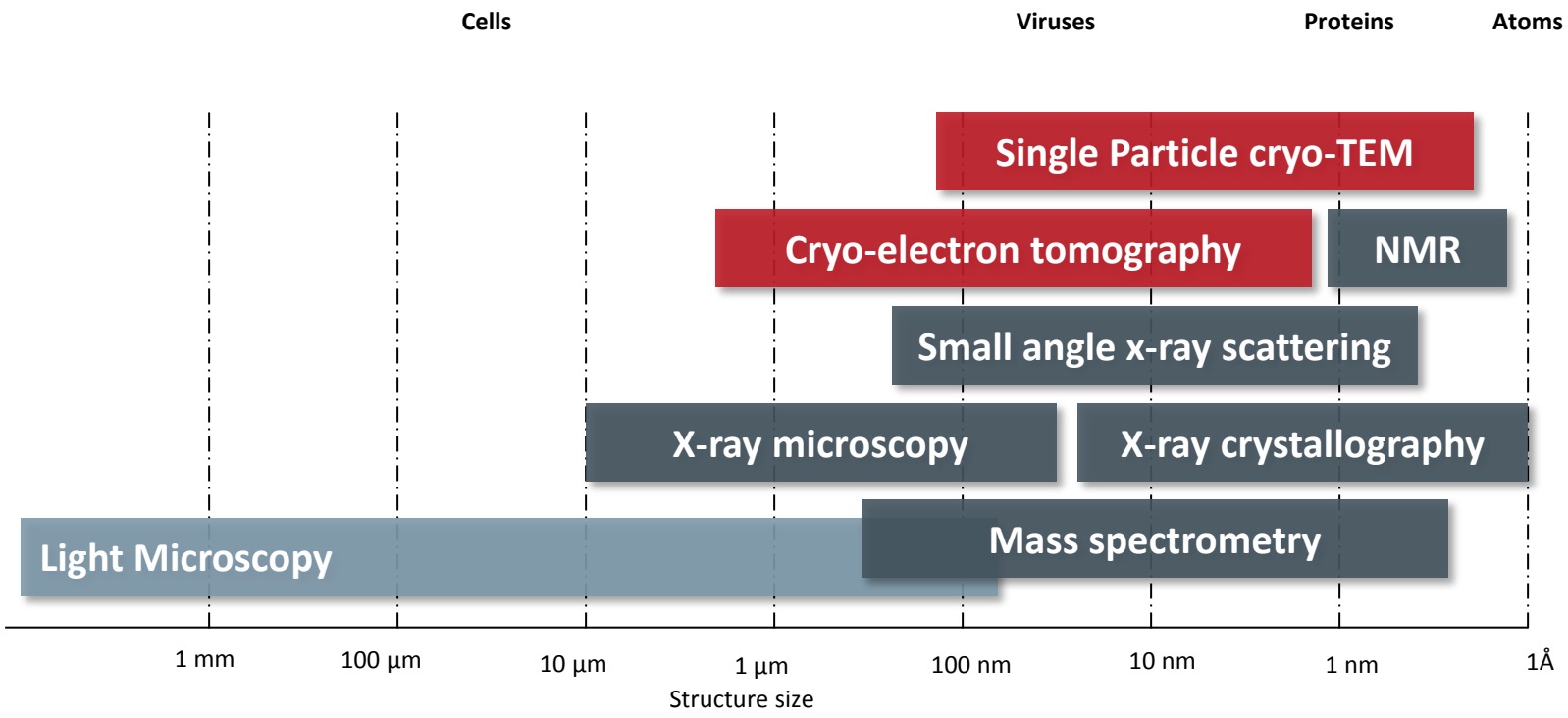
Doxorubicin: Drug packaged Liposomes. Tomography is used for verification of drug packaging

- Imaging membrane protein complexes
  - ➢ their role as receptor/donor for drugs/drug carriers



Sub-nanometer resolution structure of the intact T. thermophilus proton-driven ATP synthase – W. Lau, J. Rubinstein, DATA BANK (EMDB) / 5335

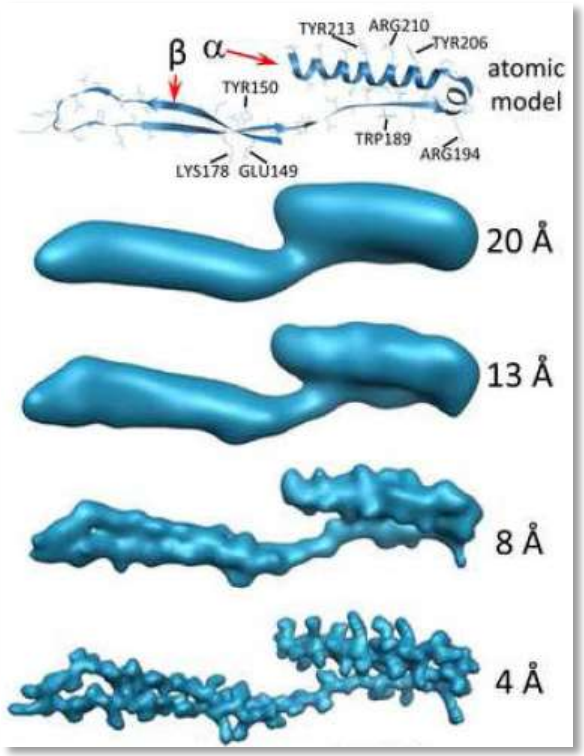
Comparison of main structural biology techniques

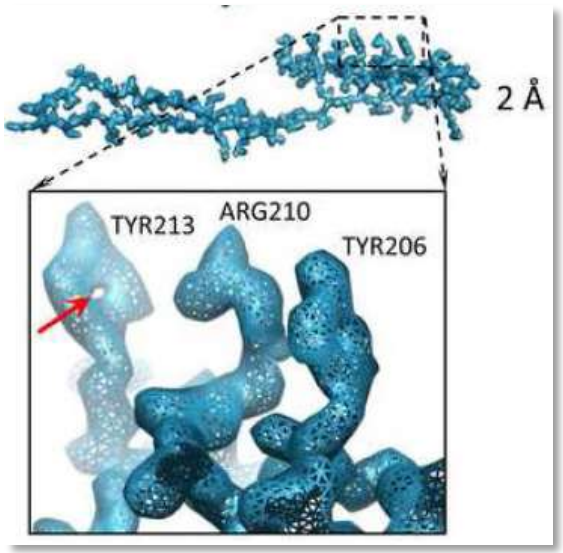


Single Particle Resolution – Why?

Secondary Structure Elements at different resolutions

Segment extracted from the atomic model of HK97 capsid protein. An alpha-helix and a beta-hairpin joined together by a loop and filtered to different resolutions.





At **4Å resolution**, strands in the **b-hairpin** begin to separate, the pitch of the **a-helix** becomes visible and **bulky side chains** can start to be seen.

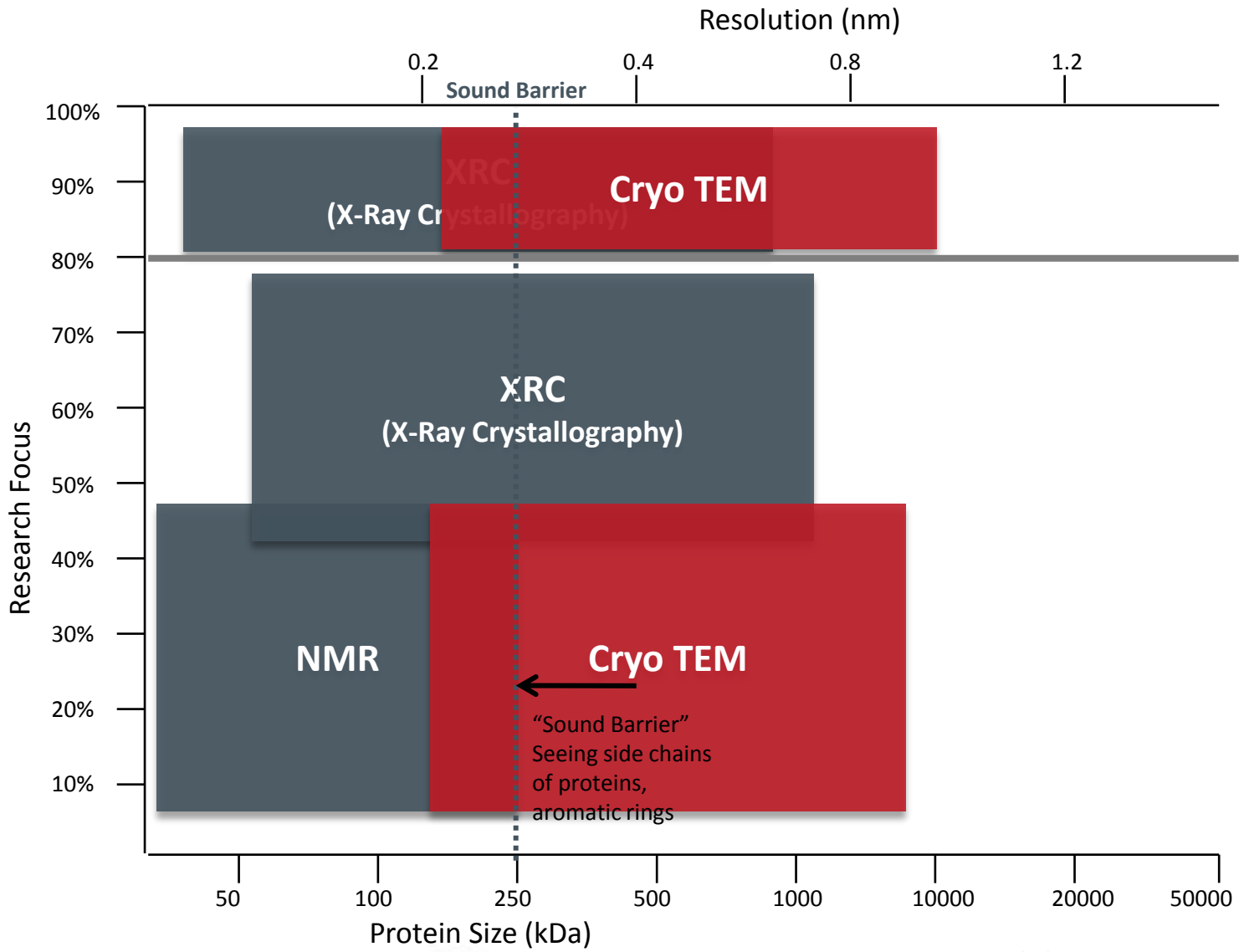
At **2Å resolution**, the hole in each **aromatic ring** is resolved (red arrow).

Towards atomic resolution structural determination by single-particle cryo-electron microscopy
Current Opinion in Structural Biology, Volume 18, Issue 2, April 2008, Pages 218-228 Z **Hong Zhou**

Structural biology research landscape

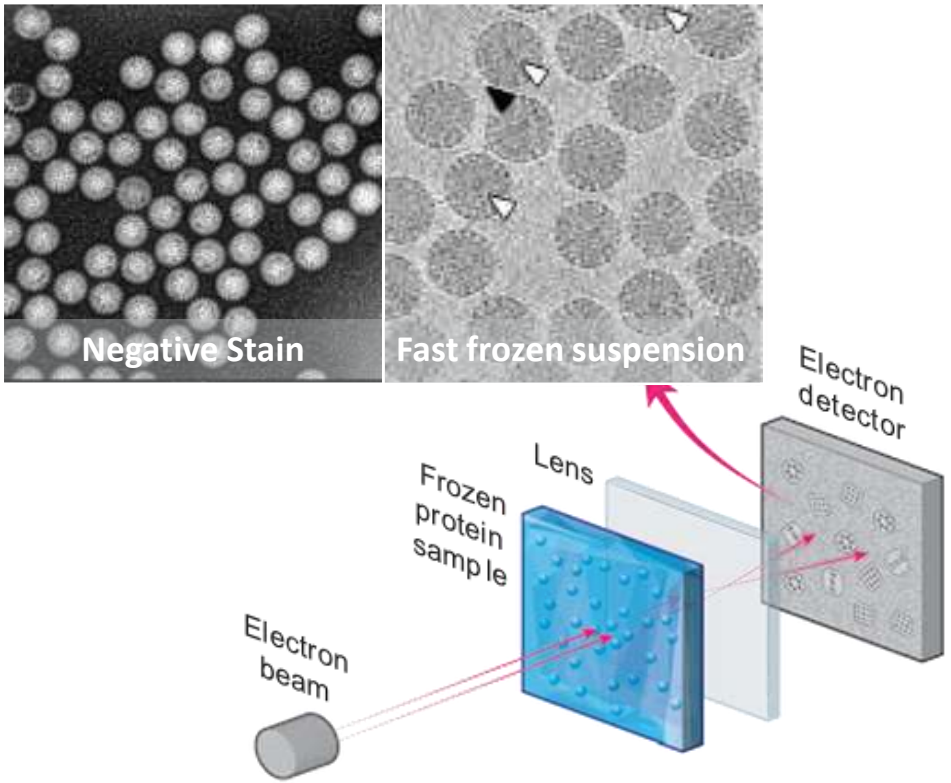
Membrane bound Proteins
Mostly crystalline, not always soluble

Cytosolic Proteins
Soluble but also capable of crystallization



What Is Cryo-TEM?

- preserves sample in the fastest and best possible way
- observes sample closer to natural state
- minimized artifacts compared to chemical fixation
- faster time to data



from E. Callaway; Nature, vol 525, p.172-174

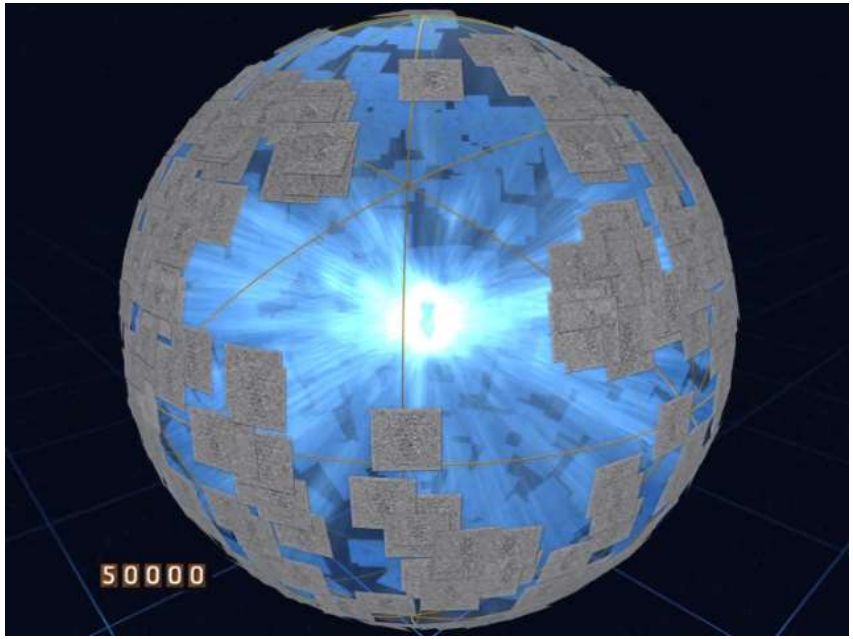
Cryo-TEM Techniques: Single Particle Analysis

3D Reconstruction from 2D Images

- Observe nature close to the natural state
- No artifacts from fixation or staining
- Prevents radiation damage
- Fix fast dynamical biological processes
- Ideal for smaller non-pleomorphic specimens

Single Particle Analysis

Proteins in solution



Animations courtesy of Max Planck Institute of Biochemistry, Martinsried, Germany

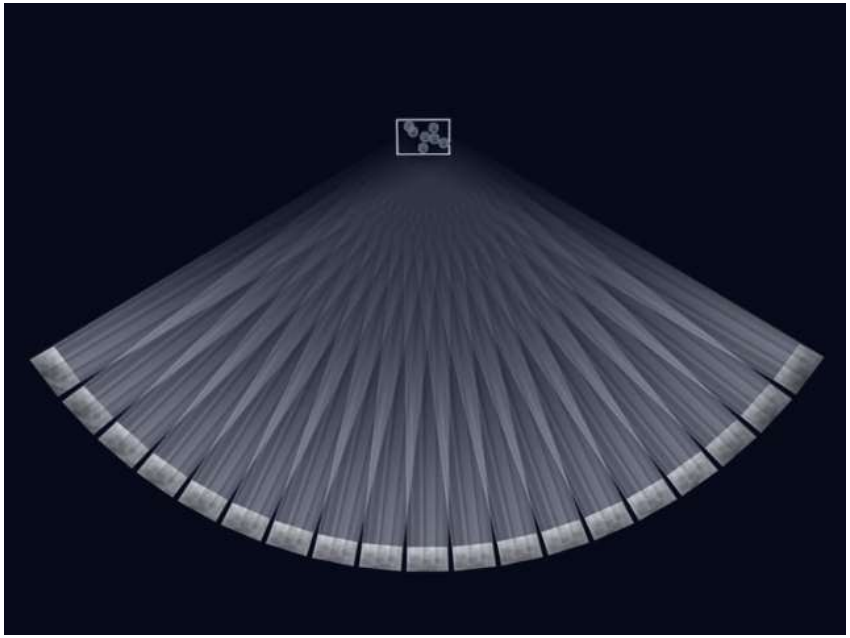
Cryo-TEM Techniques: Cryo-ET

3D Reconstruction from 2D Images

- Observe nature close to the natural state
- No artifacts from fixation or staining
- Prevents radiation damage
- Fix fast dynamical biological processes
- Ideal for larger, pleomorphic specimens

Tomography

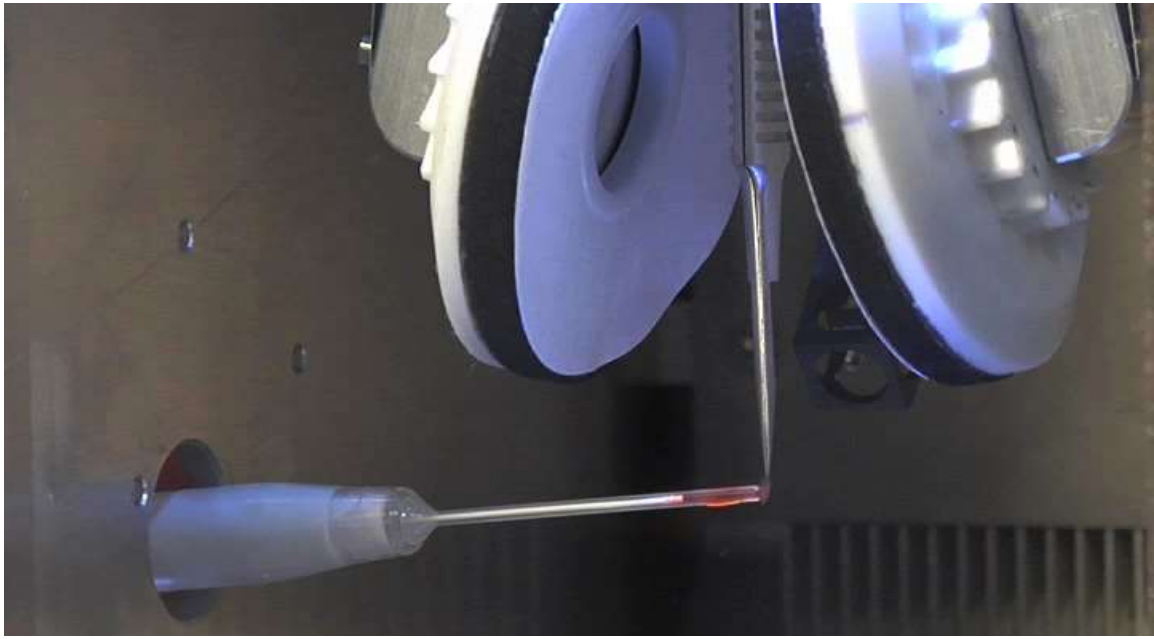
Virus in solution



Animations courtesy of Max Planck Institute of Biochemistry, Martinsried, Germany

Cryo-TEM Samples: Plunge-freezing

- Avoid harsh staining which may change the structure of your sample
- Stabilization of sample by rapid freezing of sample in liquid ethane to form vitreous ice
- Sample will stay stable in hydrated state in vacuum

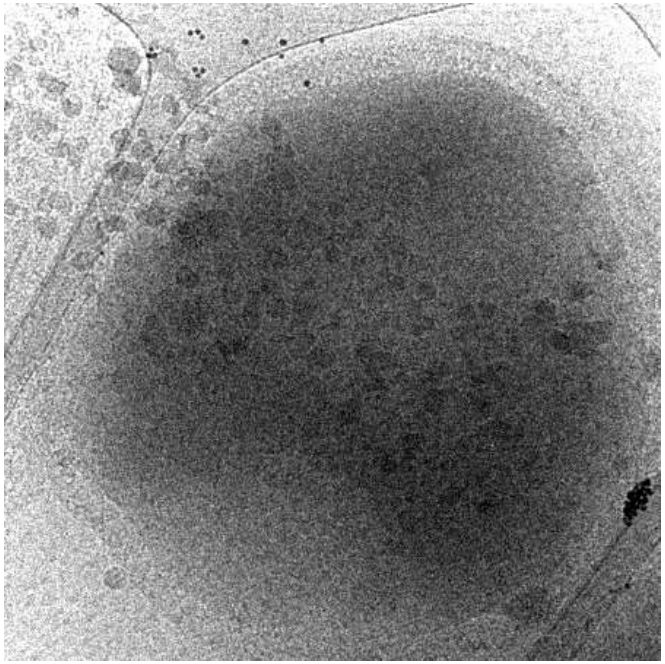


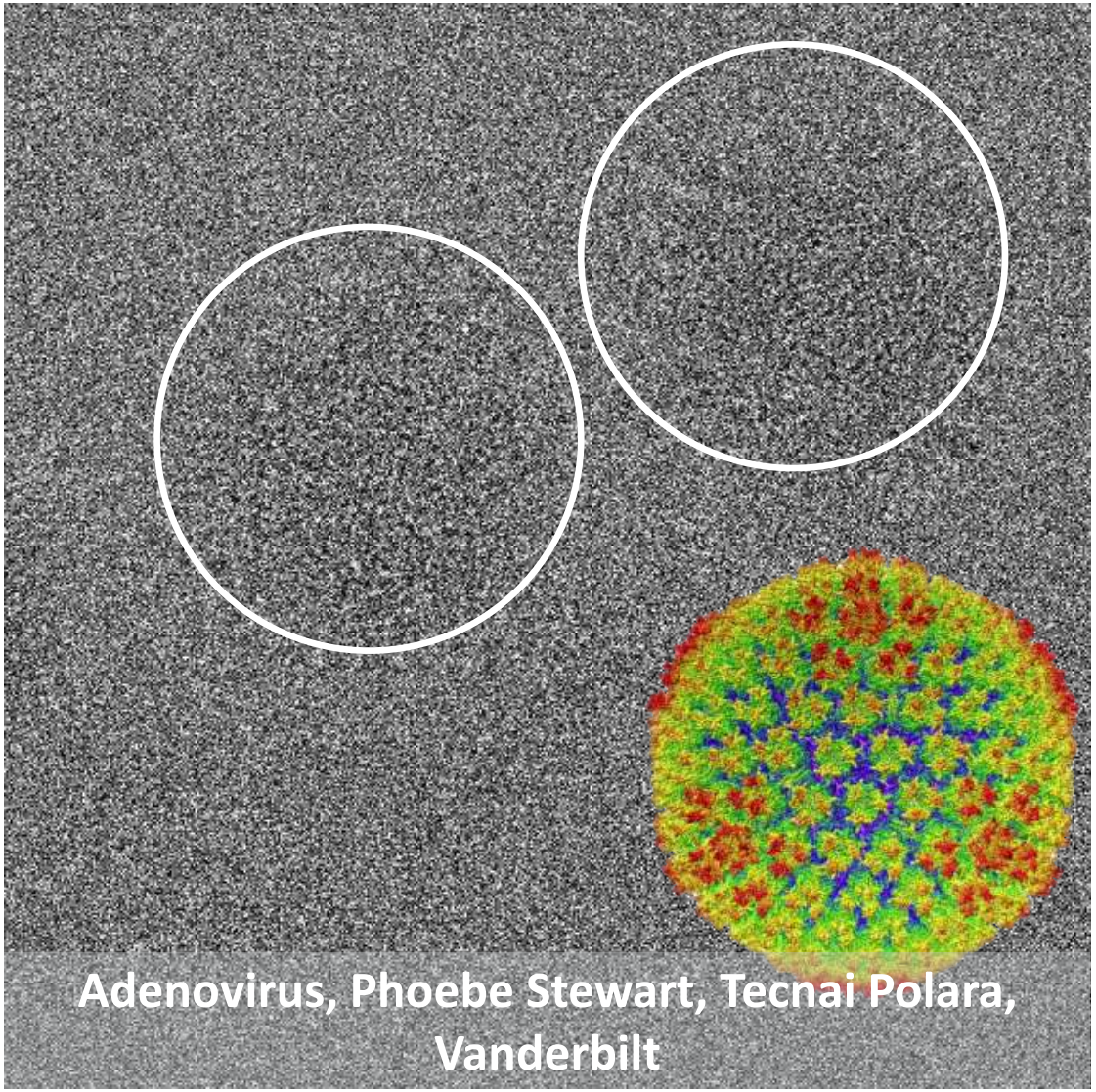
Cryo-TEM Samples: the challenges

#1: Inherent contrast problem

#2: Radiation sensitivity

Irreversible damage occurs with electron Dose of 10-50 e/Å^2





Adenovirus, Phoebe Stewart, Tecnai Polara, Vanderbilt

Nature Sept. 2015

THE REVOLUTION WILL NOT BE CRYSTALLIZED

MOVE OVER X-RAY CRYSTALLOGRAPHY. CRYO-ELECTRON MICROSCOPY IS KICKING UP A STORM IN STRUCTURAL BIOLOGY BY REVEALING THE HIDDEN MACHINERY OF THE CELL.

BY EWEN CALLAWAY

In a basement room, deep in the bowels of a steel-clad building in Cambridge, a major insurgency is under way.

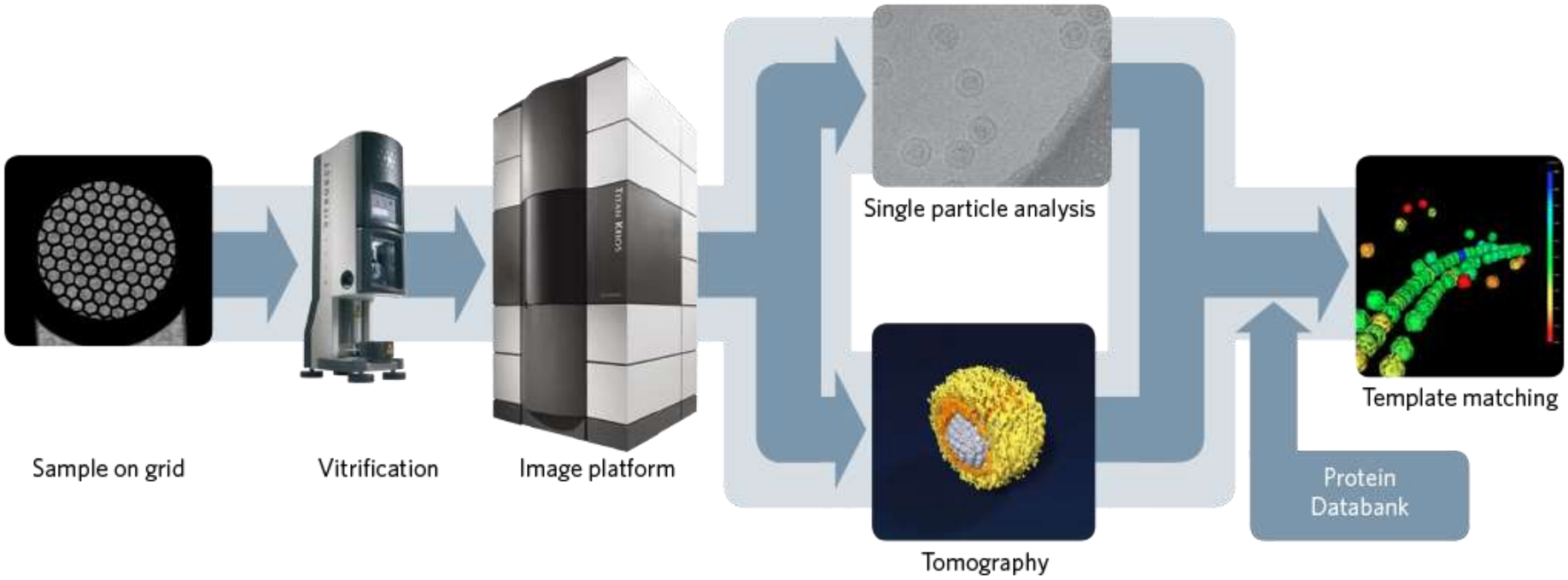
A hulking metal box, some three metres tall, is quietly beaming terabytes' worth of data through thick orange cables that disappear off through the ceiling. It is one of the world's most advanced cryo-electron microscopes: a device that uses electron beams to photograph frozen biological molecules and lay bare their molecular shapes. The microscope is so sensitive that a shout can ruin an experiment, says Sjors Scheres, a structural biologist at the UK Medical Research Council Laboratory of Molecular Biology (LMB), as he stands dwarfed beside the £3-million (US$7.7-million) piece of equipment. "The UK needs many more of these, because there's going to be a boom," he predicts.

In labs around the world, cryo-electron microscopes such as this one are sending tremors through the field of structural biology. In the past three years, they have revealed exquisite details of protein-making ribosomes, quivering membrane proteins and other key cell molecules.

20 Years Ago...

- Working in the dark
- Recording on film
- Mainly negative stain RT work
- Manual work with exotic specimen holders
- 3D work only possible by aligning stacks of 2D images manually

and today: cryo-EM workflow



Imaging platform today...

- Brighter electron sources
- Superior optics
- Superior vacuum
- Autoloading of samples (Artica & Krios)
- Contrast enhancement
- Breakthrough cameras

- **24/7 operation** without operator on site
- Fully digital microscopy
- **Automated sample handling**
- Fully **automated 3D analysis** (SPA and Tomography)

Talos Arctica

Full Automation for dedicated SPA and Tomography

- Unattended high data throughput, reduced time-to-result.
  - Robotic sample handling (auto-loading up to 12 samples)
  - Auto filling of LN2 for continuous platform operation
  - Automated data acquisition through tailor made SW
- Excellent data quality
  - Optimized for 80-200kV
  - C-TWIN objective lens
  - Contamination free sample loading
  - Increased sample life time (>24 hours)

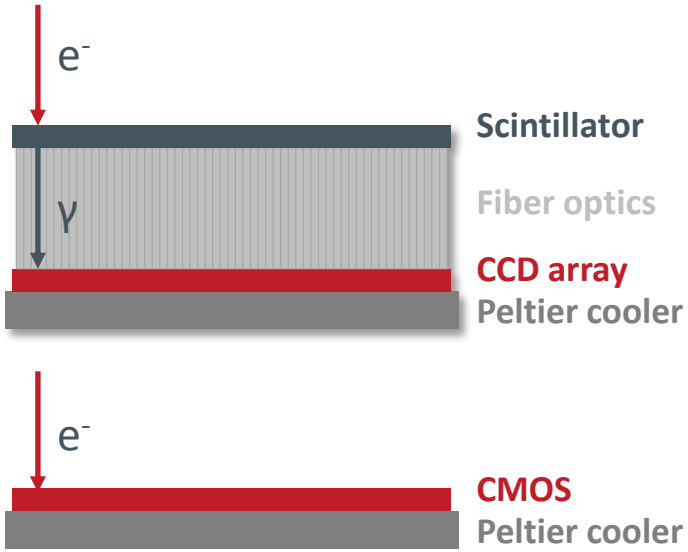
Titan Krios G2

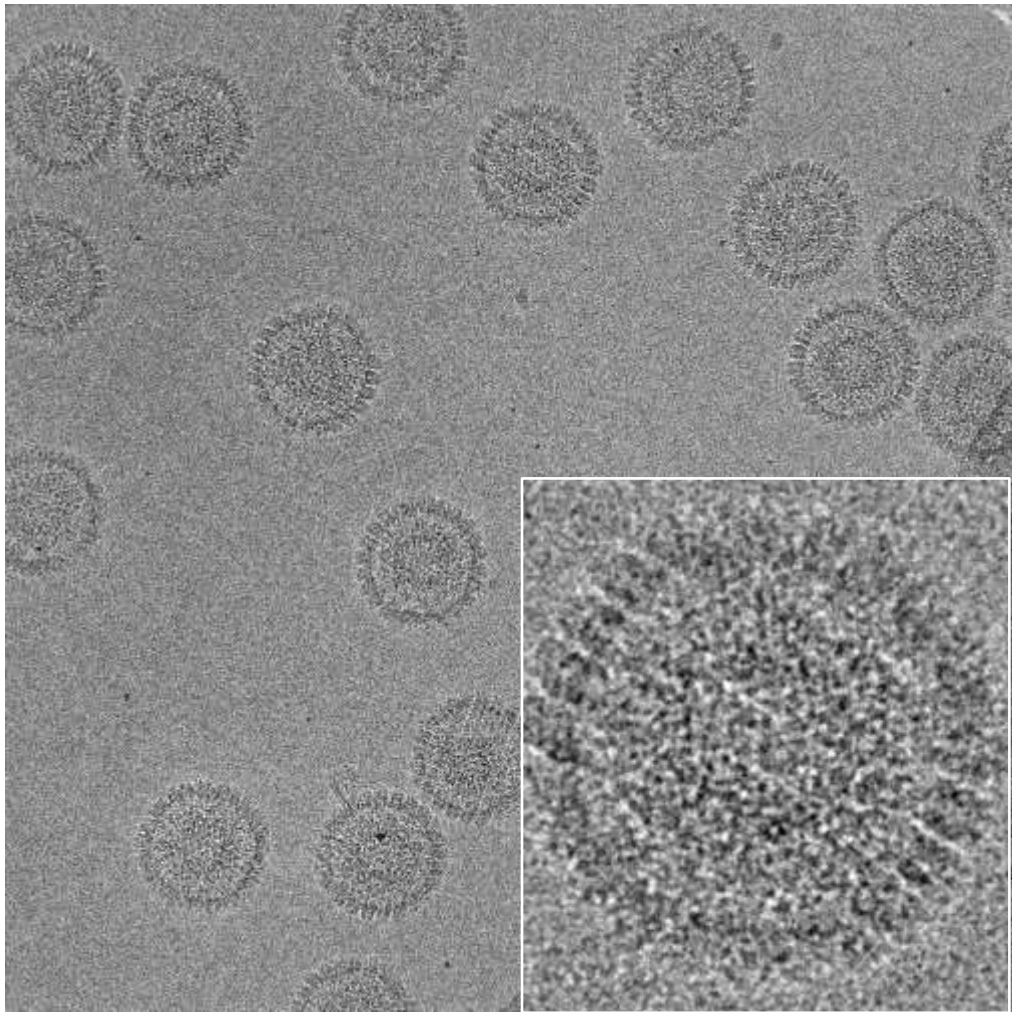
The ultimate fully automated high end cryo-TEM for SPA and Tomography

- Rock stability, based on proven Titan technology
  - Mechanical: Wide column
  - Electrical: Constant power lenses
  - Environmental: “Boxed” design
- Robotic sample handling
- Loading of 12 samples
- LN_2 Autofill
- Parallel illumination
- Optimized for Structural (Cellular) Biology applications: Cryo tomography and SPA
- Dual axis tilt holder (+/- 70 degrees) enabling dual axis tomography
- Daylight operation

Direct electron detectors

- Higher sensitivity, signal/noise ratio and resolution than CCD cameras

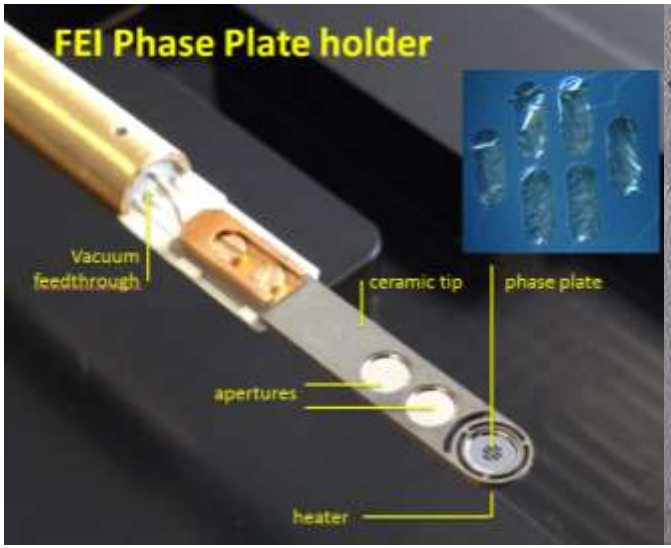


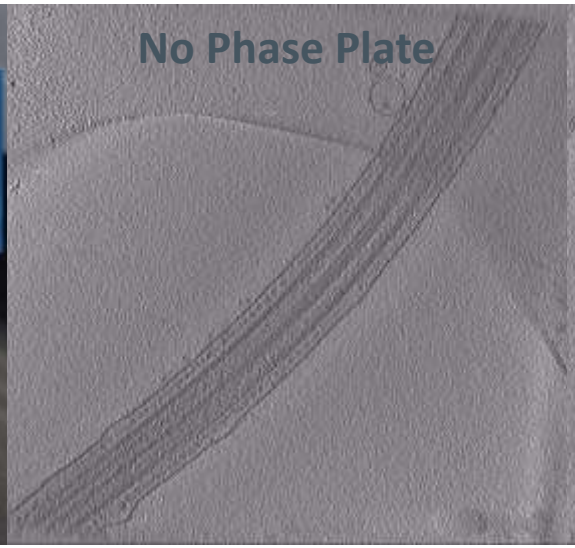


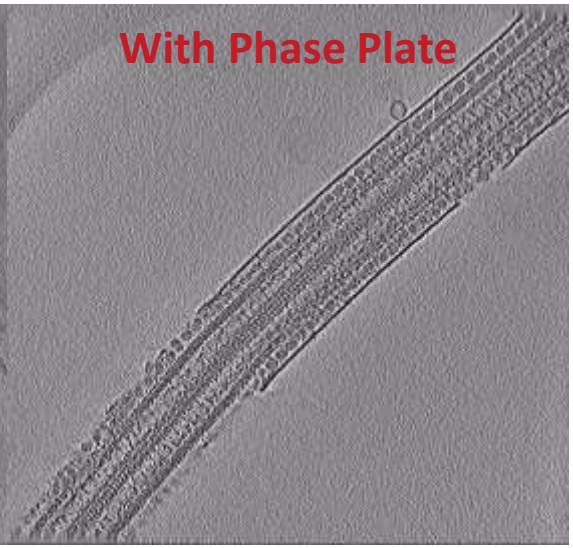
Herpes simplex virus imaged on a FEI TITAN KRIOS using the Falcon II. The capsids are 1250 Å in diameter.
Courtesy of Anastasia Aksyuk, William Newcomb, and Alasdair Steven, NIAID, NIH.

FEI Volta Phase Plate

- Low resolution contrast can be increased by applying high defocus (typically 4 micron), but as a consequence, there is contrast loss at high resolution
- Alternative: change the phase contrast mechanism by shifting the relative phases of the scattered and unscattered electrons by 90 degrees
  - Less electron dose needed
  - Unseen structures are visible, thicker samples can be imaged
  - Long **life time** (6 months) and **stability** for extended, long time cryo-TEM experiments
- **patented** technology

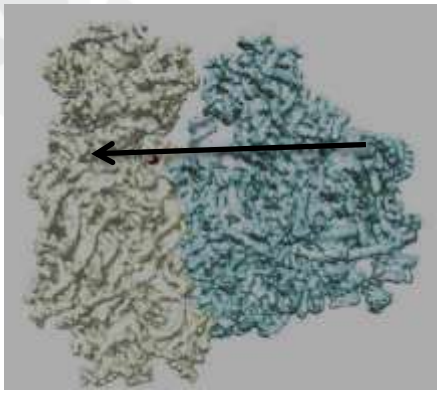


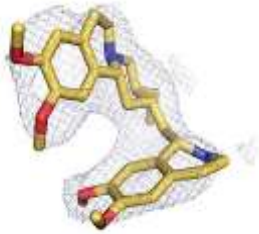


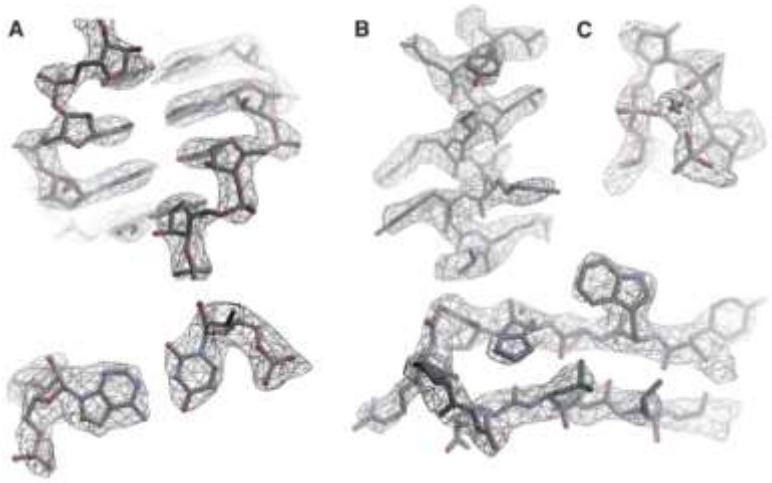


Worm sperm; Courtesy M. Khoshouei, MPI Martinsried

Ribosome (2014):



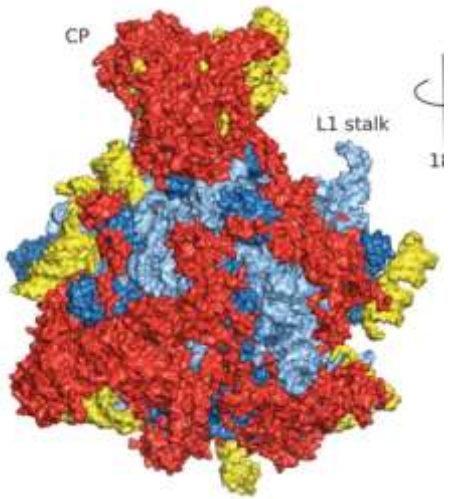




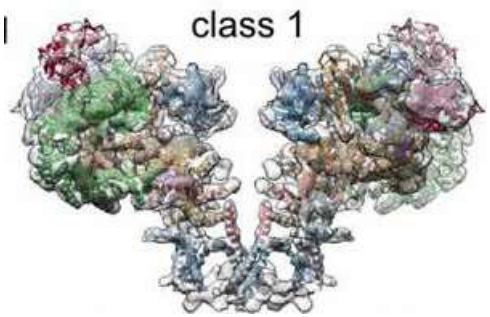
Full *de novo* model built

Hussain T, et al. **Cell** (2014) 159 pp. 597-607
Bischoff L, et al. **Cell Rep**. (2014) 9 pp. 469-475
Arenz S, et al. **Molecular Cell** (2014)
Brown A, et al. **Science** (2014) 346 pp. 718-722
Greber BJ, et. Al. **Nature** (2014)
Shao S, et al. **Molecular Cell** (2014) 55 pp. 880-890
Voorhees RM, et al. **Cell** (2014) 157 pp. 1632-1643
Wong W, et al. **eLife** (2014) 3
Fernandez IS, **Cell** (2014) 157 pp. 823-831
Amunts A, **Science** (2014) 343 pp. 1485-1489
Greber BJ, et al. **Nature** (2014) 505 pp. 515-519 (cover)

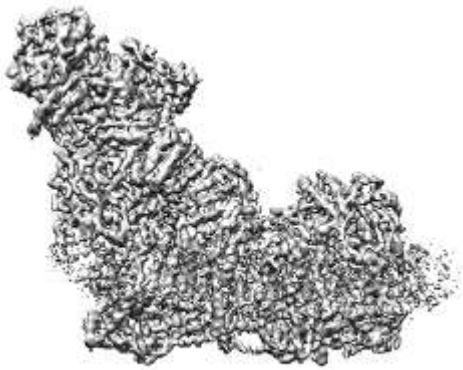
Routinely ≤4Å



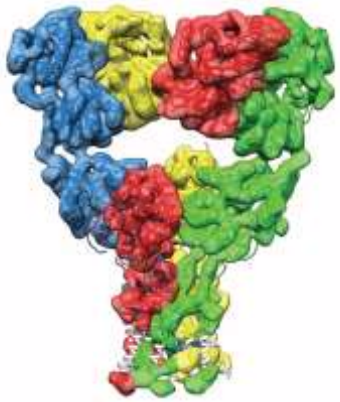
Membrane proteins:

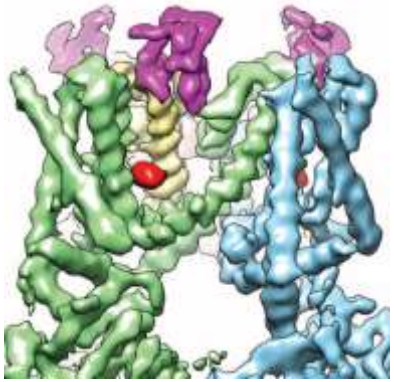


Ryanodine receptor (2.2MD)



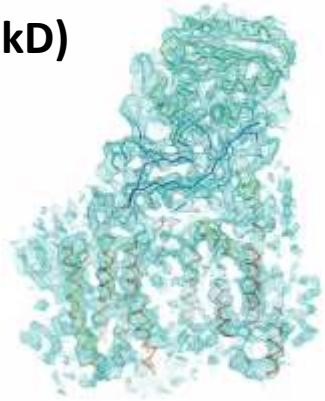
Complex I (1MD)





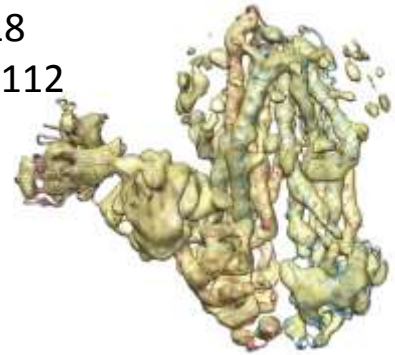
TRPV1 (300 kD)

Glutamate receptor (460kD)



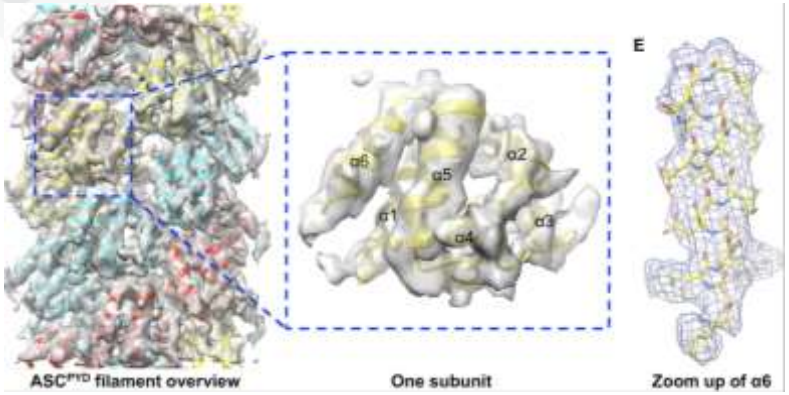
γ-secretase (170 kD)

Kutti R. Vinothkumar, et al. **Nature** (2014) 515 pp. 80-84
Erhu Cao, et al. **Nature** (dec 2013) 504 pp. 113-118
Maofu Liao, et al. **Nature** (dec 2013) 504 pp. 107-112
JungMin Kim, et al. **Nature** (2014)
Joel R. Meyerson, et al. **Nature** (2014)
Peilong Lu, et al. **Nature** (2014)
Rouslan G. Efremov, et al. **Nature** (2014)

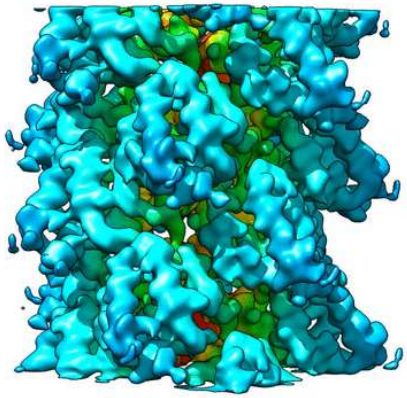


ABC-transporter (135 kD)

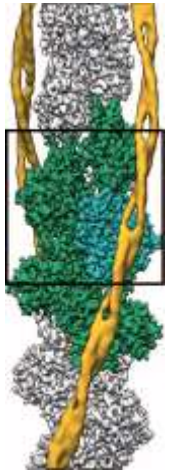
Filaments:



Inflamasomes

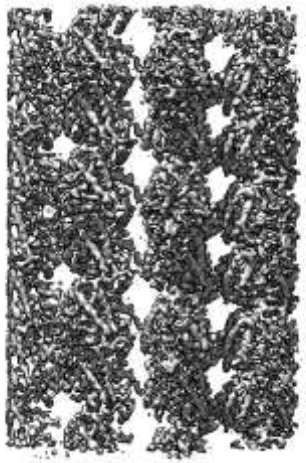


MAVS filament



Actin

Lu A, et al. **Cell** (2014) 156 pp. 1193-1206
Alushin GM et al. **Cell** (2014) 157 pp. 1117-1129
Wu B, et al. **Molecular Cell** (2014) 55 pp. 511-523
Von der Ecken J, et al. **Nature** (2014)
Egelman group: actin, **Structure Cell** (accepted)



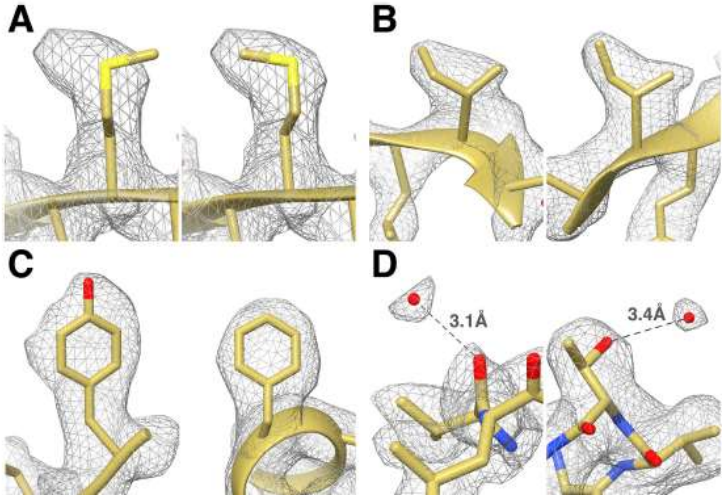
Microtubules

Pushing the resolution

2.8 Å resolution reconstruction of the *Thermoplasma acidophilum* 20S proteasome using cryo-electron microscopy

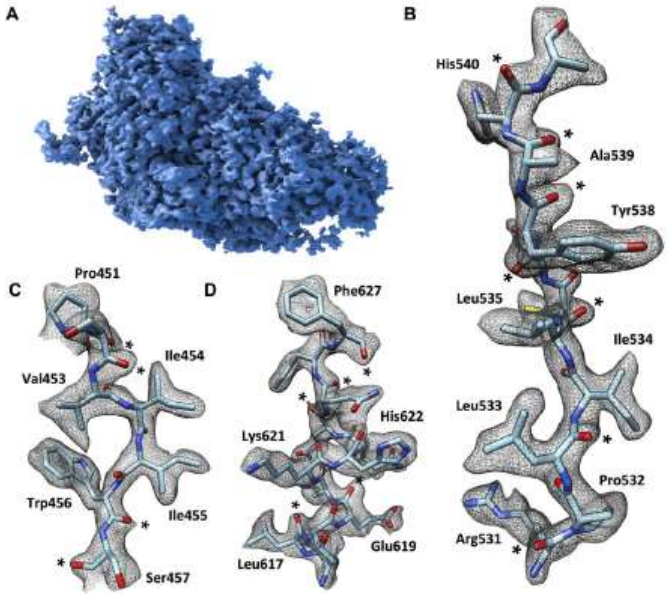
Melody G Campbell[1,2†], David Veesler[1,2,3†], Anchi Cheng[1,2,4], Clinton S Potter[1,2,4], Bridget Carragher[1,2,4*]

Campbell et al. eLife 2015;4:e06380. DOI: 10.7554/eLife.06380



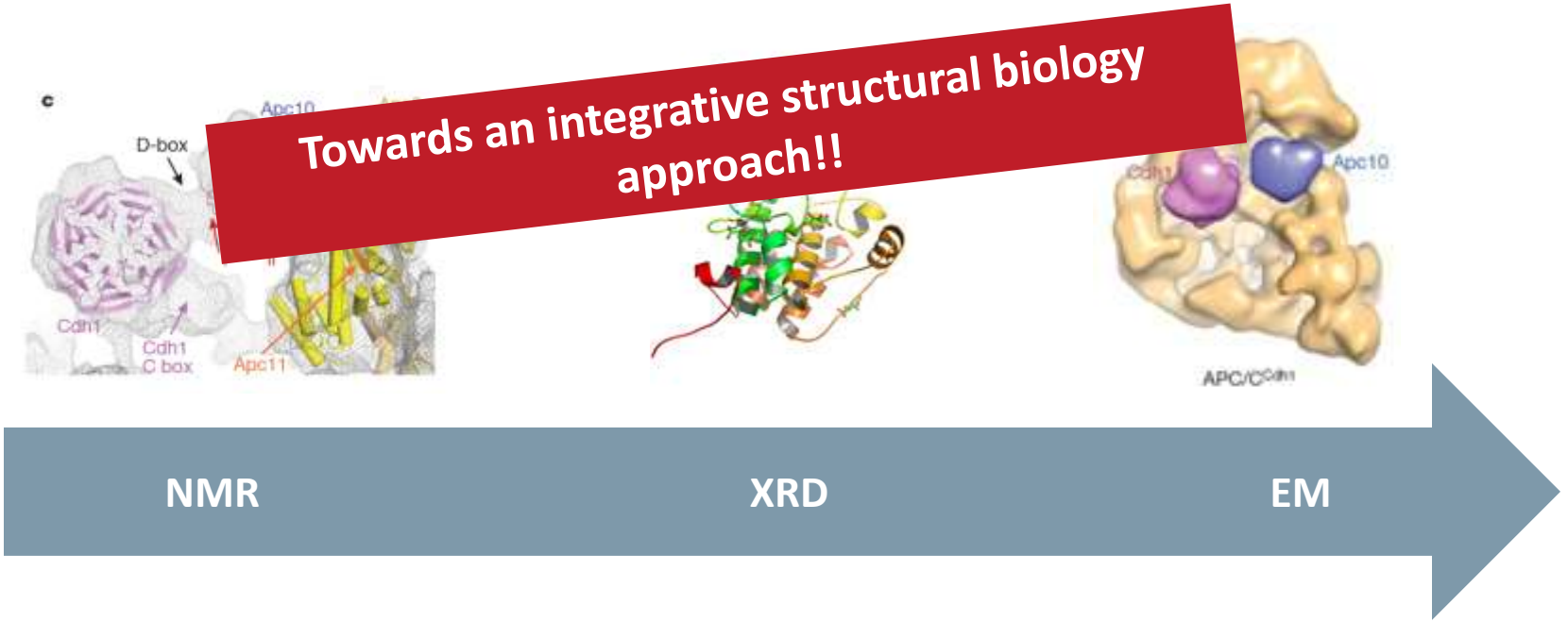
2.2 Å resolution cryo-EM structure of β-galactosidase in complex with a cell-permeant inhibitor

Alberto Bartesaghi,[1*] Alan Merk,[1*] Soojay Banerjee,[1] Doreen Matthies,[1] Xiongwu Wu,[2] Jacqueline L. S. Milne,[1] Sriram Subramaniam[1†]

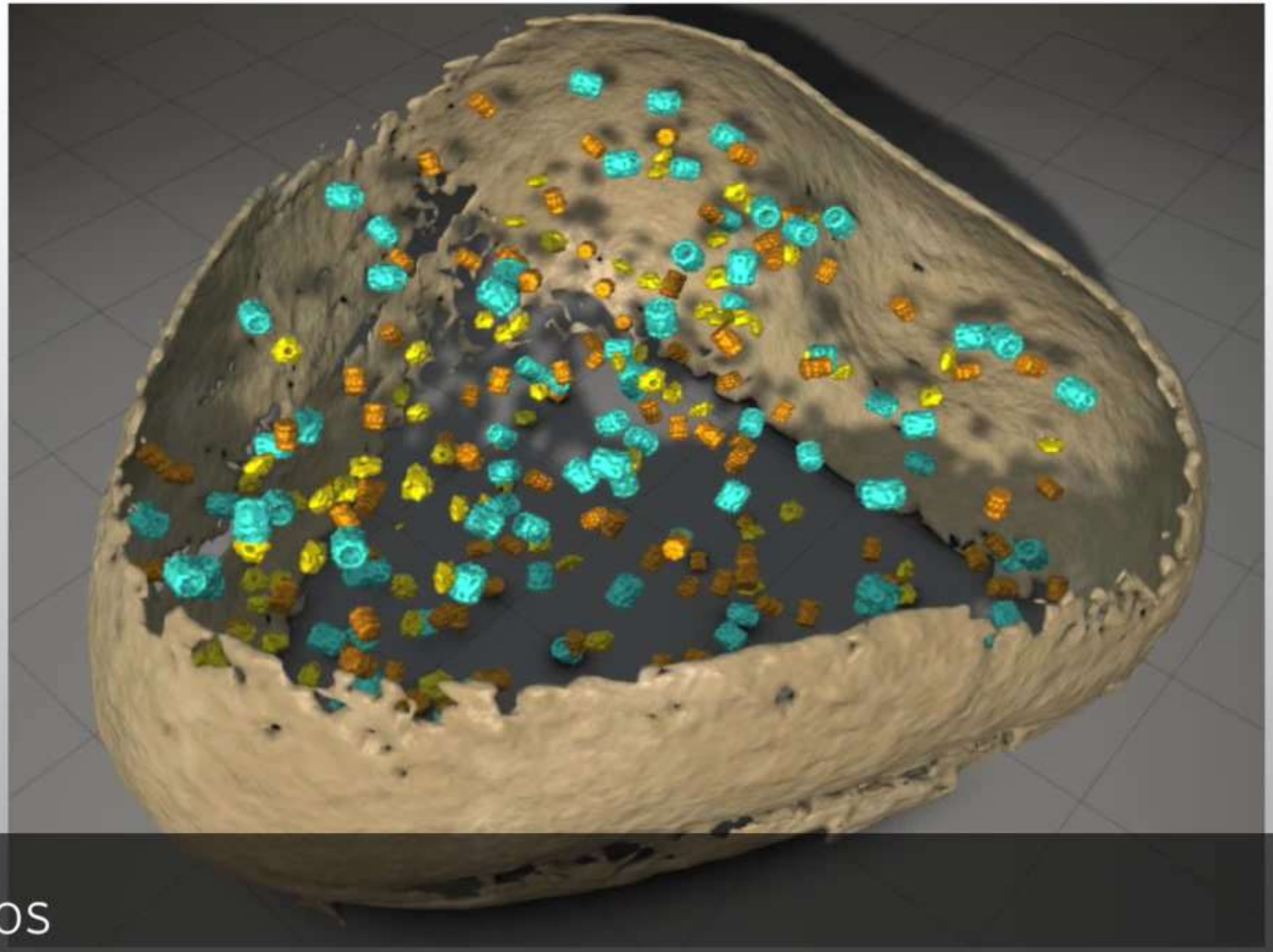


Complementarity of XRD, NMR and Cryo-TEM

- 2 – 20 Angström information required to understand function of dynamic biological complexes
- Hybrid methodology using NMR, XRD and Cryo-TEM are often required to answer biological questions



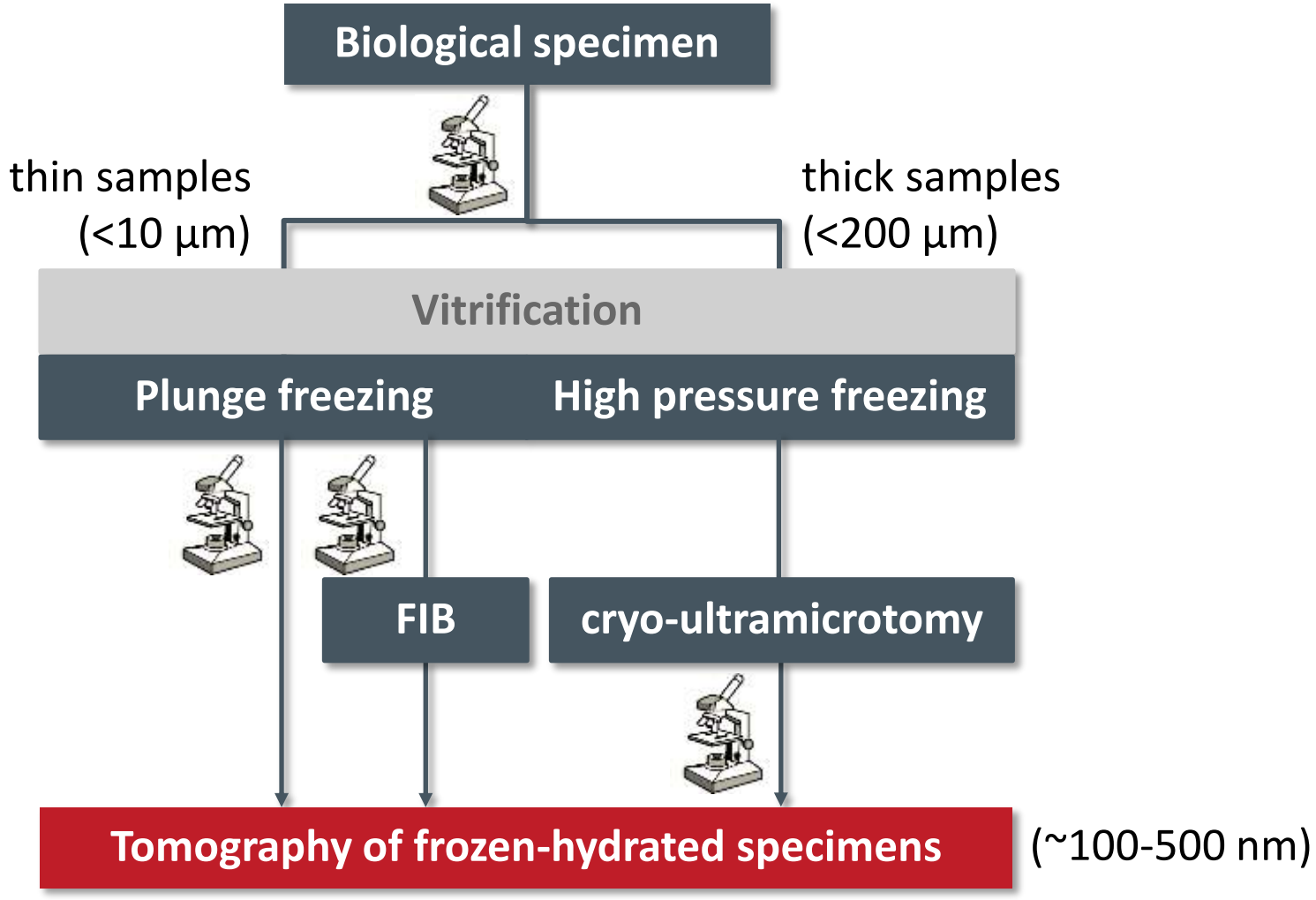
Template matching



Argos

Selected volume tomography (towards in-situ structural biology)

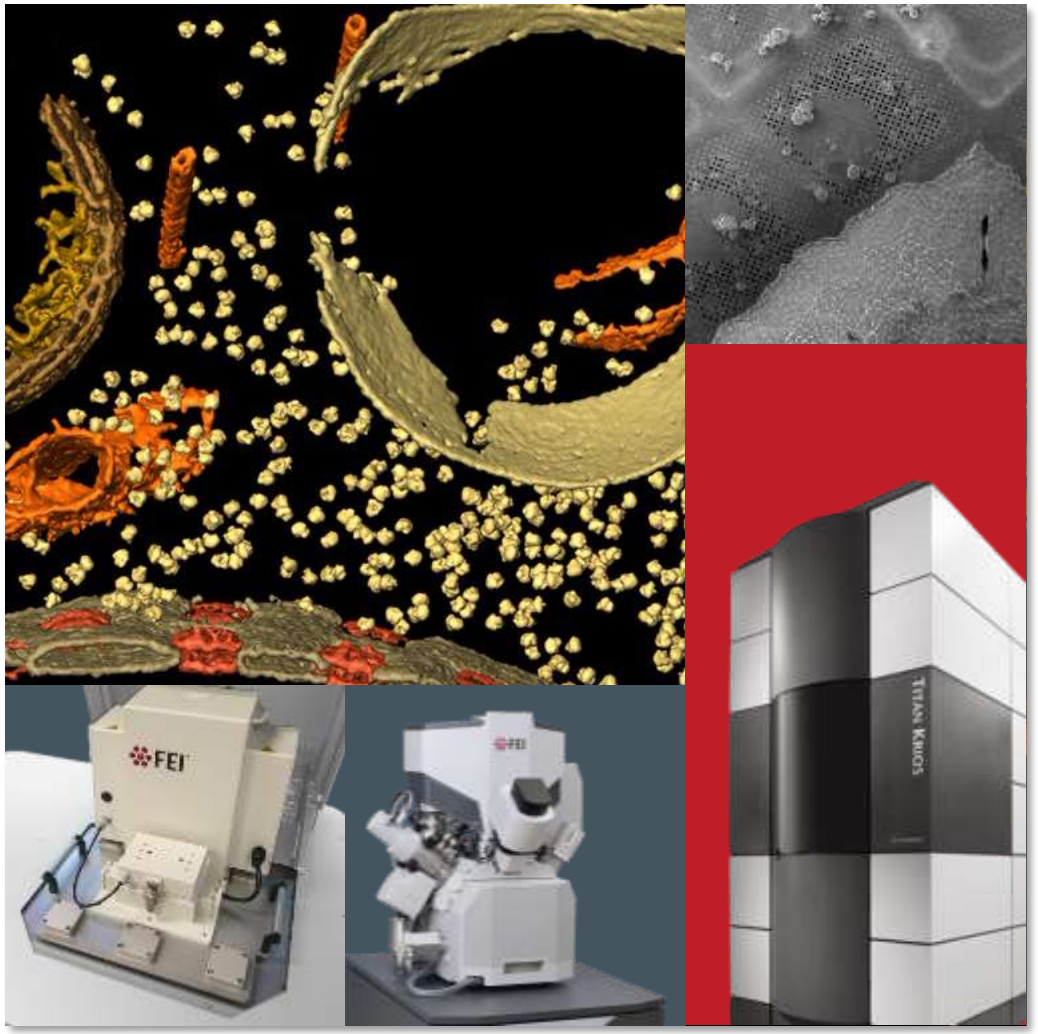
Cryo-electron-tomography workflow



Adapted from Rigort et al., Methods in Cell Biology Vol. 111 (2012)

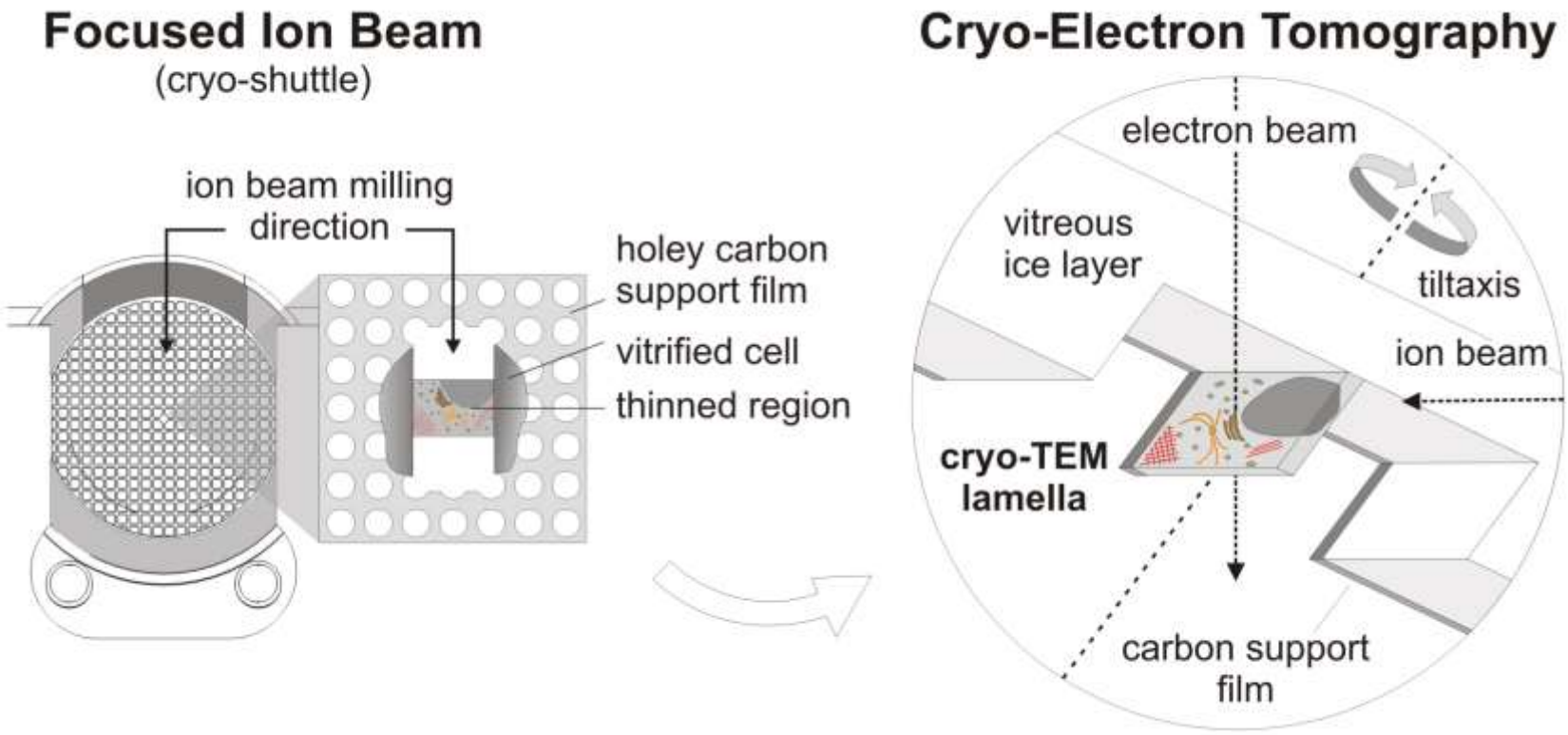
Experimental steps

- Cell culture on EM grids
- Plunge freezing
- Autogrid mounting
- Cryo-LM
- Cryo-FIB milling
- Cryo-electron tomography
- Reconstruction & visualization with AMIRA



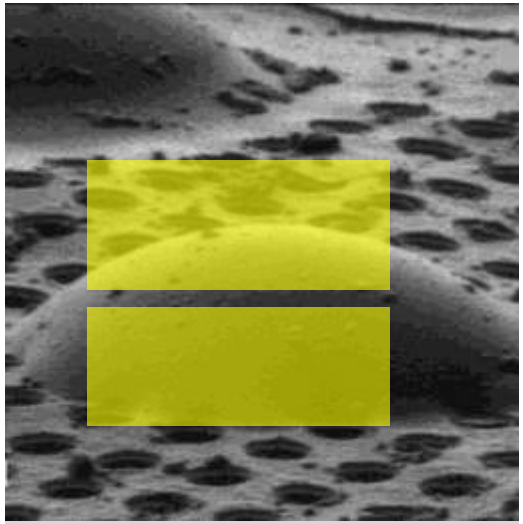
Courtesy of W. Baumeister and J. Plitzko, MPI for Biochemistry

Cryo-FIB milling

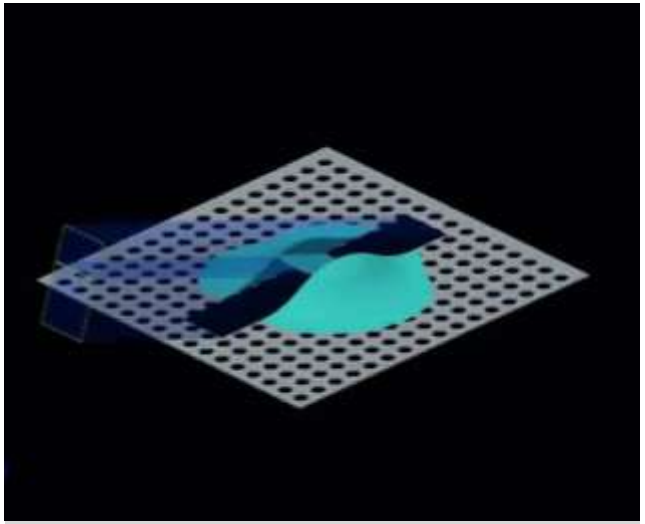


Rigort et al., Proc Natl Acad Sci USA (2012) Mar 20;109(12):4449-54.

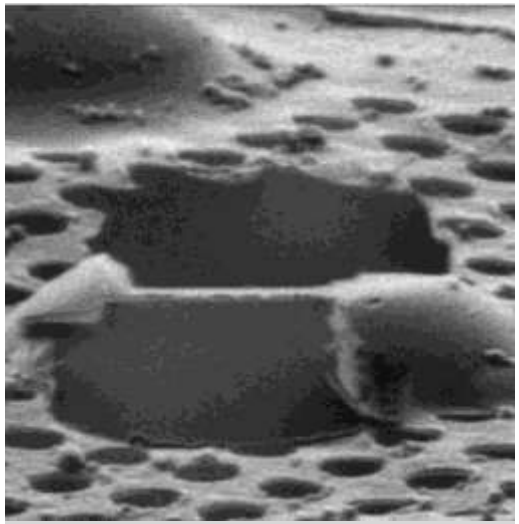
Cryo-FIB milling of lamella



Define milling area (ion beam image)



A lamella of 80-350 nm supported by the remains of the cell is created



Resulting lamella (ion beam image)

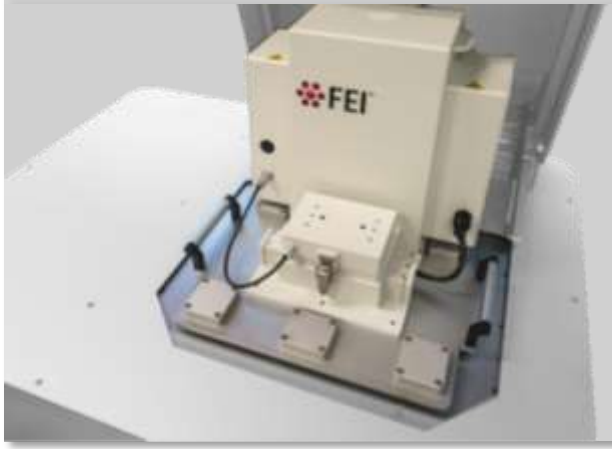
Courtesy of W. Baumeister and J. Plitzko

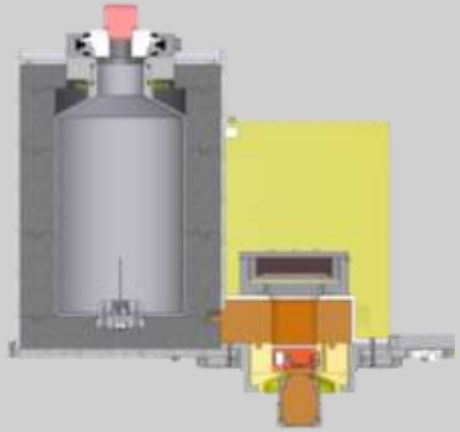
Max Planck Institute of Biochemistry Martinsried, Germany

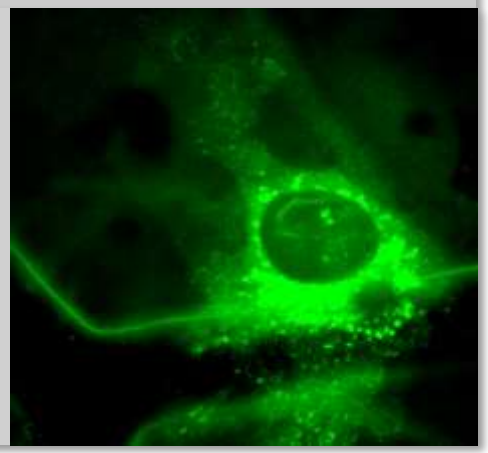
Cryo-light microscopy

CorrSight cryo

- **No LN_2 pump** needed
- Up to **2 grid positions** in a fixed geometry
- Compatible with **40x/0.9 NA** objective
- **No condensation** / frost
- Samples pre-mounted on shuttles for **quick and safe exchange**
- Works with **all CorrSight imaging modes**: transmission, widefield fluorescence, SI, spinning disk confocal

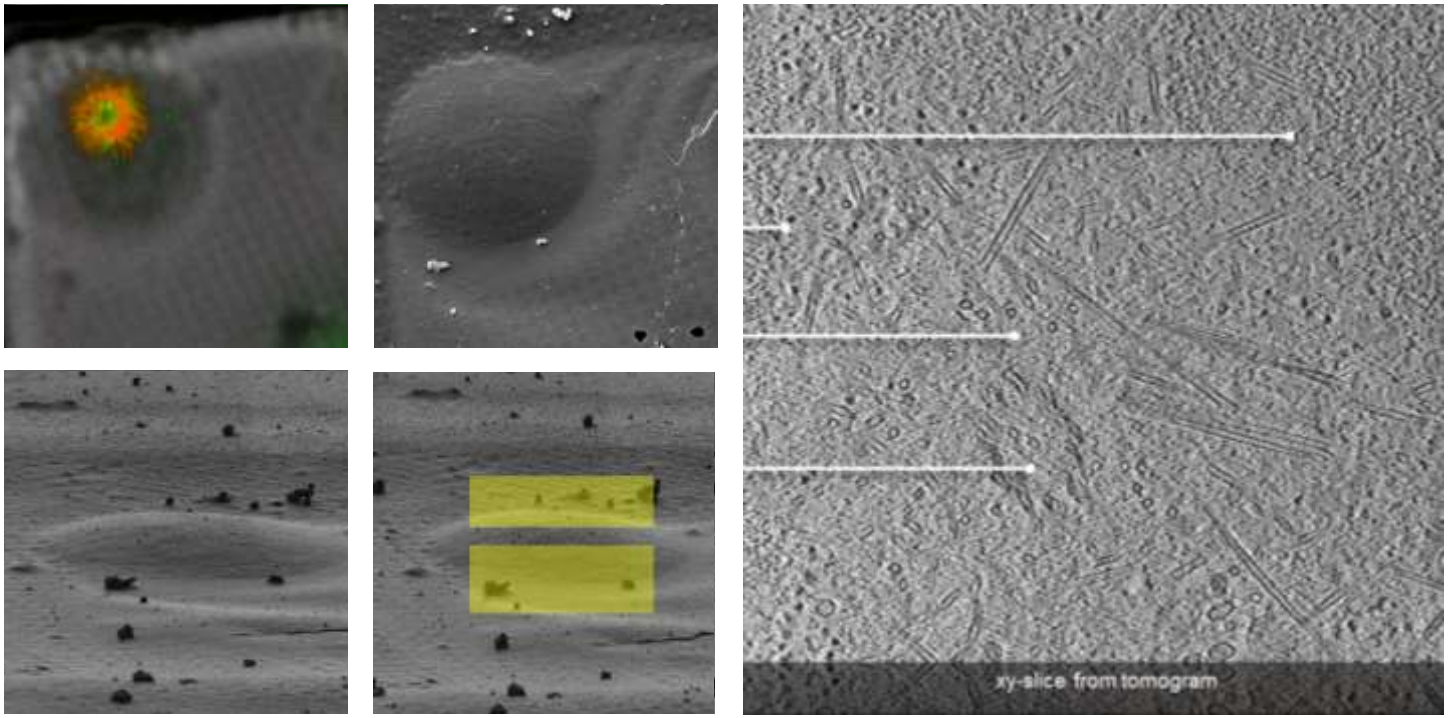






Courtesy of Dorit Hanein, Sanford Burnham

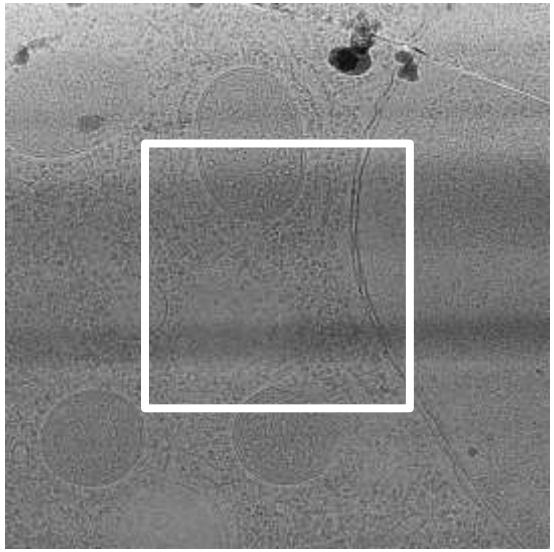
Imaging of HeLa cells: from cryo-light microscopy to cryo-TEM, through cryo-FIB-milling



Courtesy of J. Mahamid and J. Plitzko, MPI for Biochemistry

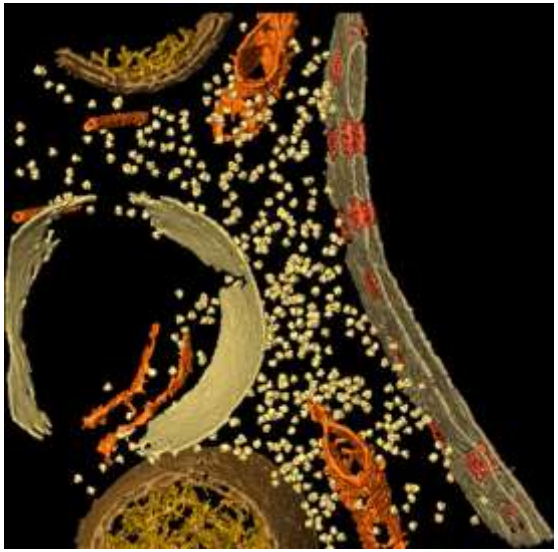
Cryo-ET of FIB-milled lamella

TEM projection



3D reconstruction

Surface rendering



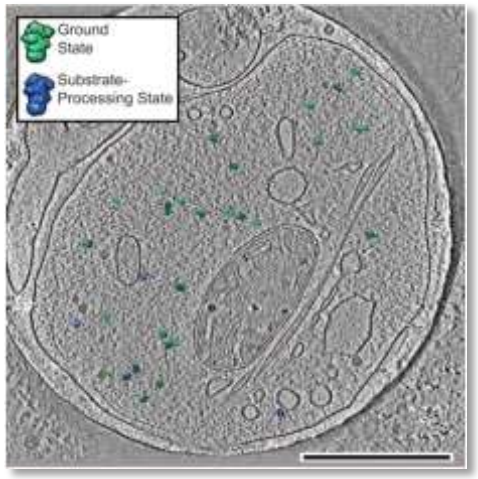
Courtesy of J. Plitzko

Max Planck Institute of Biochemistry Martinsried, Germany

Proteasomes. A molecular census of 26S proteasomes in intact neurons

Asano et al., Science. 2015 Jan 23;347(6220):439-42

“The 26S proteasome is a key player in eukaryotic protein quality control and in the regulation of numerous cellular processes. Here, we describe quantitative in situ structural studies of this highly dynamic molecular machine in intact hippocampal neurons. We used electron cryotomography with the Volta phase plate, which allowed high fidelity and nanometer precision localization of 26S proteasomes. We undertook a molecular census of single- and double-capped proteasomes and assessed the conformational states of individual complexes. [...]”

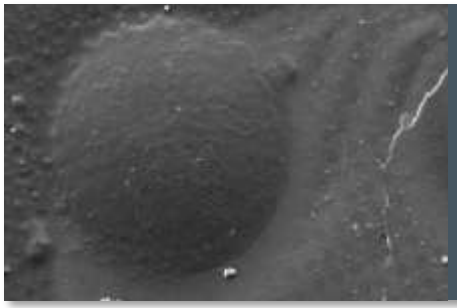


Max Planck Institute of Biochemistry Martinsried, Germany

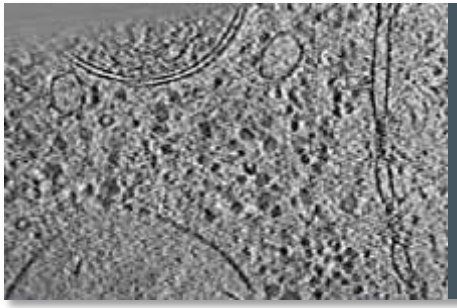
Cell biology solutions

The importance of scale

Small organisms and tissues: millimeters



Cell: ≈300 µm^3

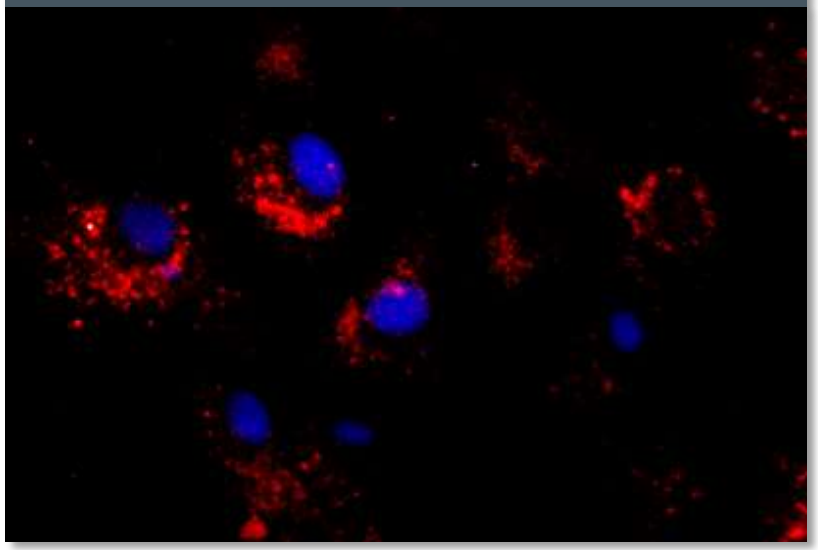


Tomogram: ≈0.4 µm^3

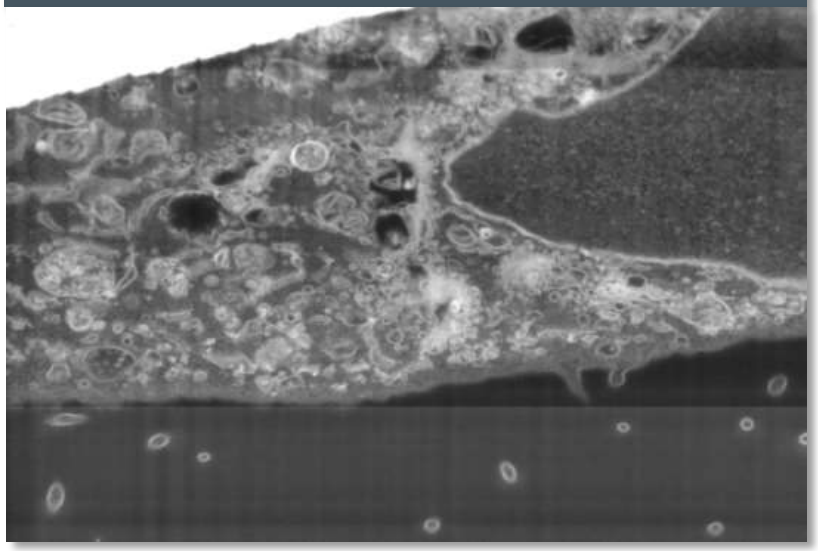
Top image: Courtesy of D. McCarthy, University College London
Middle and bottom: Courtesy of J. Mahamid, J. Plitzko and W. Baumeister, MPI for Biochemistry

Motivation for CLEM

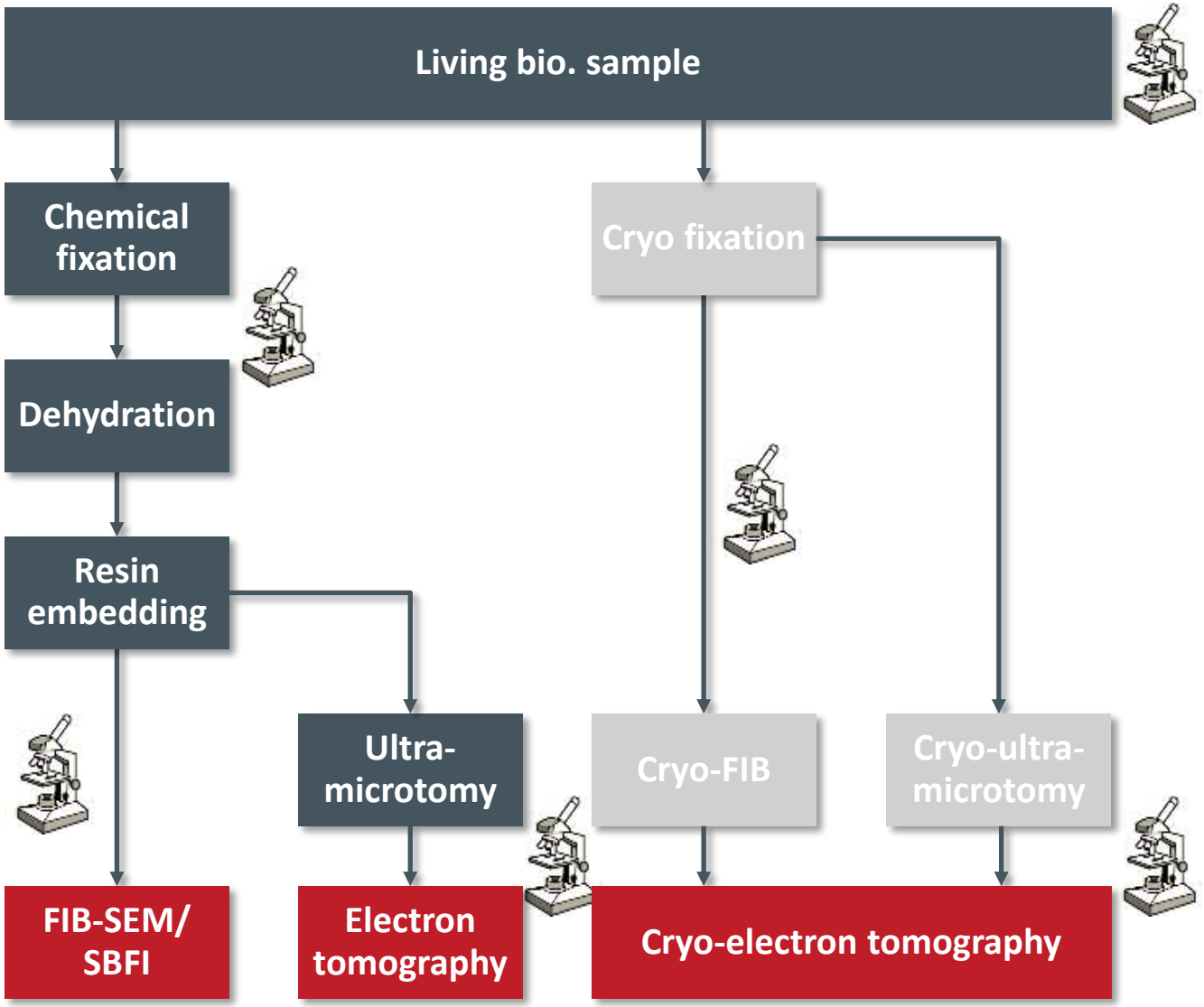
Light Microscopy
- Dynamics (e.g. live samples)
- Fluorescent probes/labels
- Limited resolution
- Large Field of View



Electron Microscopy
- Embedded/Frozen samples
- 2D/3D structural imaging
- High resolution
- Small Field of View

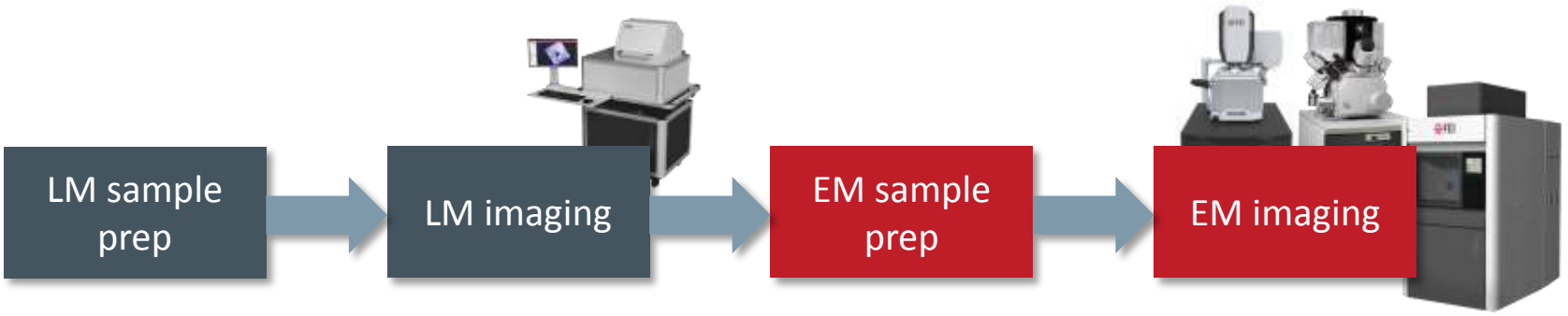


Sample preparation workflows

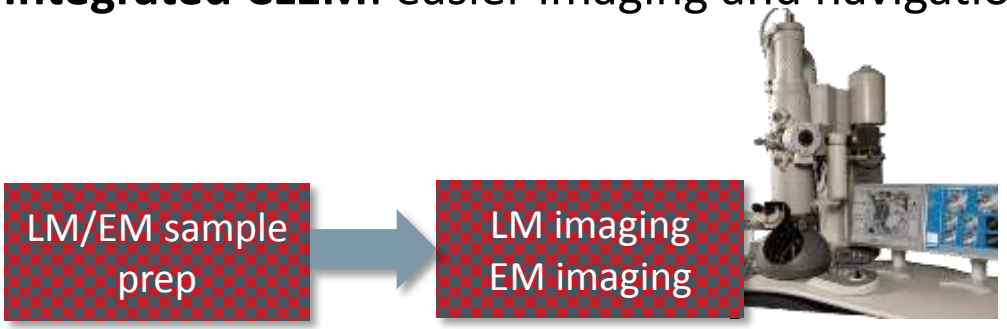


Two approaches for CLEM

Sequential CLEM: flexibility in LM imaging and EM labeling and staining



Integrated CLEM: easier imaging and navigation, maximum sample protection



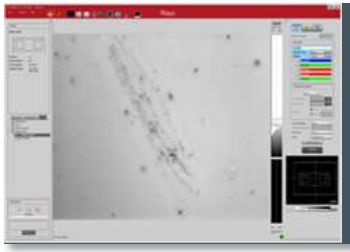
Solutions for CLEM from FEI

iCorr

- **Integrated CLEM** on dual-modality TEM

CorrSight

- Dedicated light-microscopy system for **sequental CLEM** workflow



MAPS & Amira

- MAPS: Unified software interface for navigation and acquisition on LM & SEM/SDB & TEM soon
- Amira: Visualize and register 3D LM & EM data

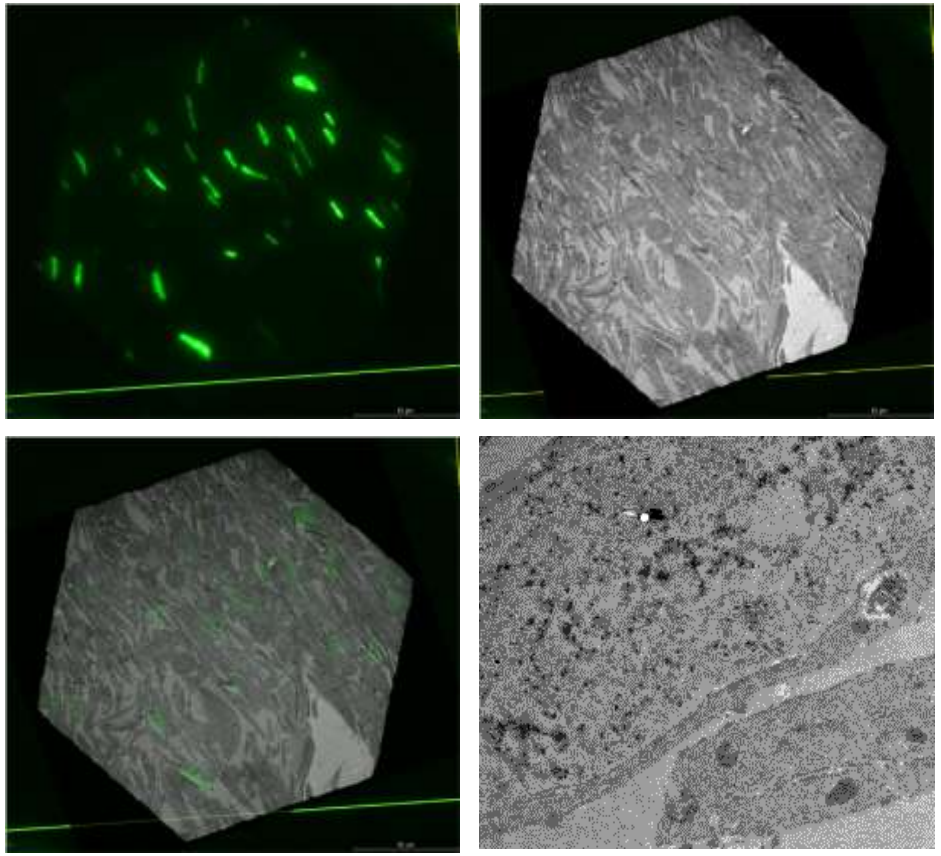
iCorr

Integrated CLEM on TEM

Tecnai T20/TF20/T12; BioTWIN/TWIN lens configurations

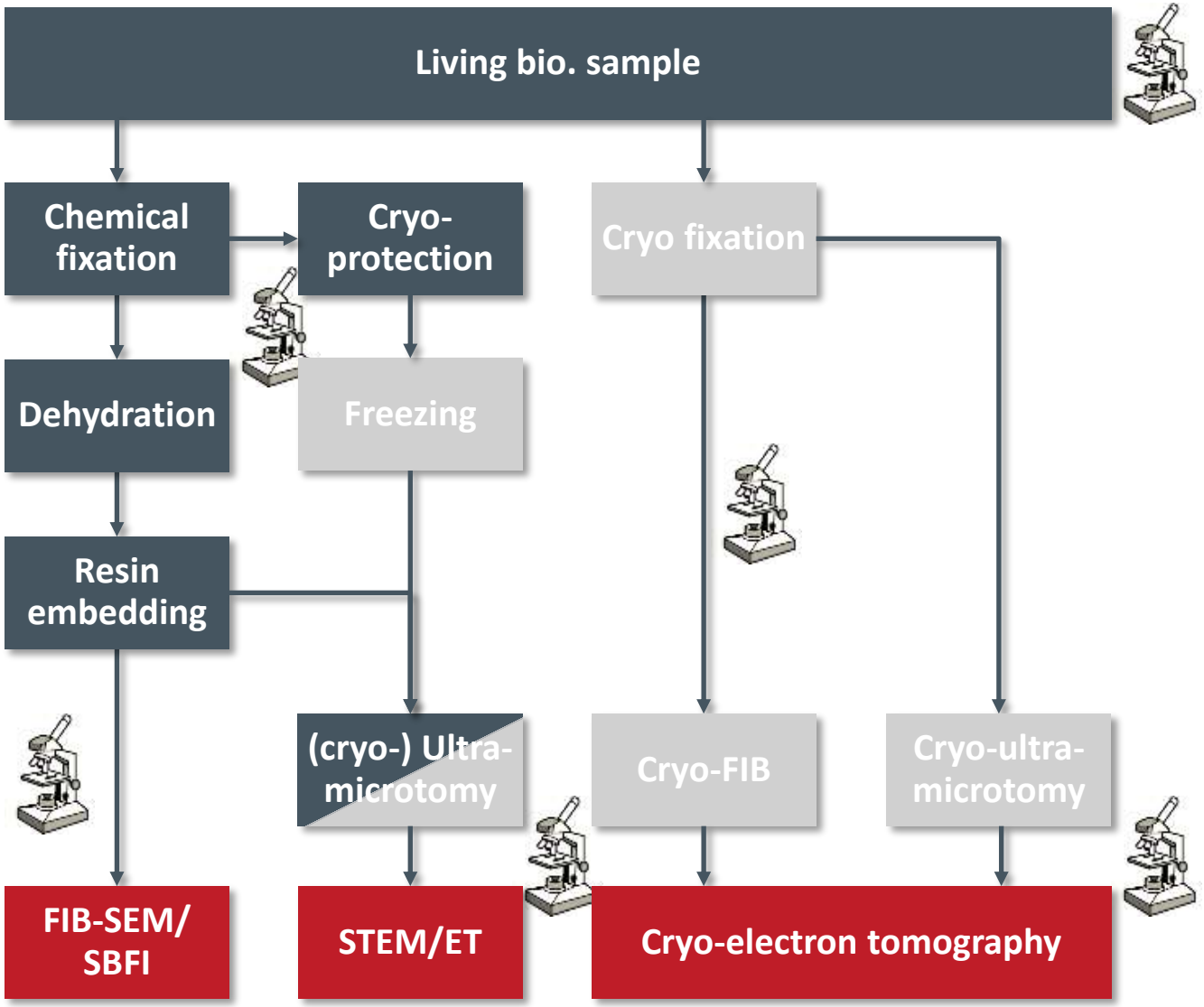
iCorr- intuitive navigation in correlative workflow

- Optimal navigation tool, fully integrated in the TEM
- Special 15x 0.5 NA objective
- Maximum sample protection, no transfer of samples between instruments
- Instant image overlay and scaling of optical and electron microscopy data

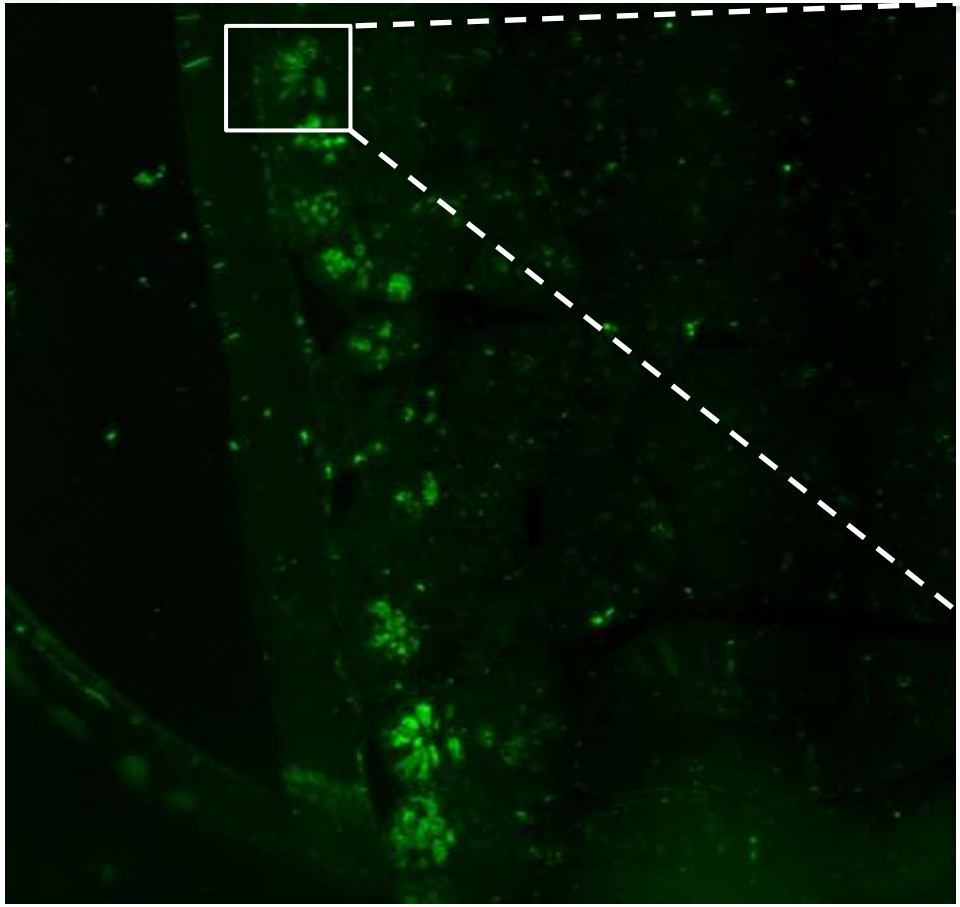


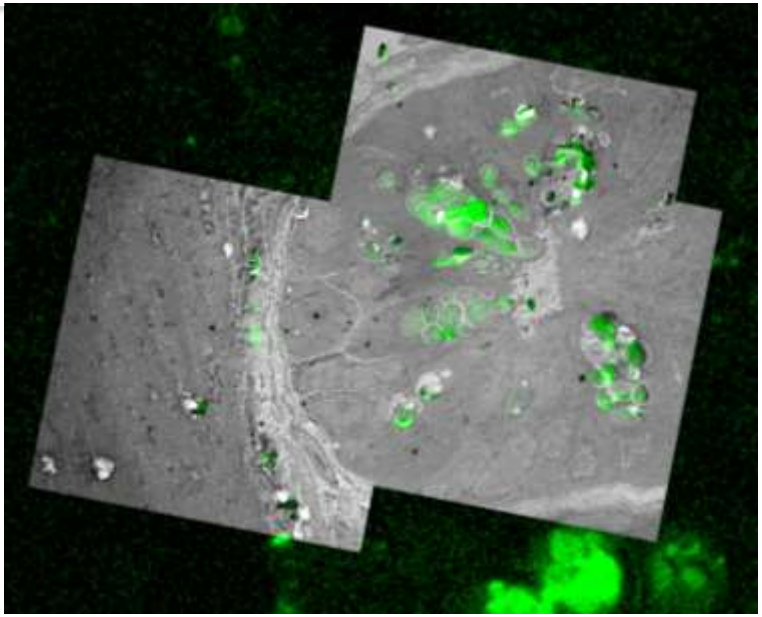
Courtesy of M. Karreman (EMBL) and E. van Donselaar (University Utrecht)

Sample preparation workflows



iCorr: Immunolabeling

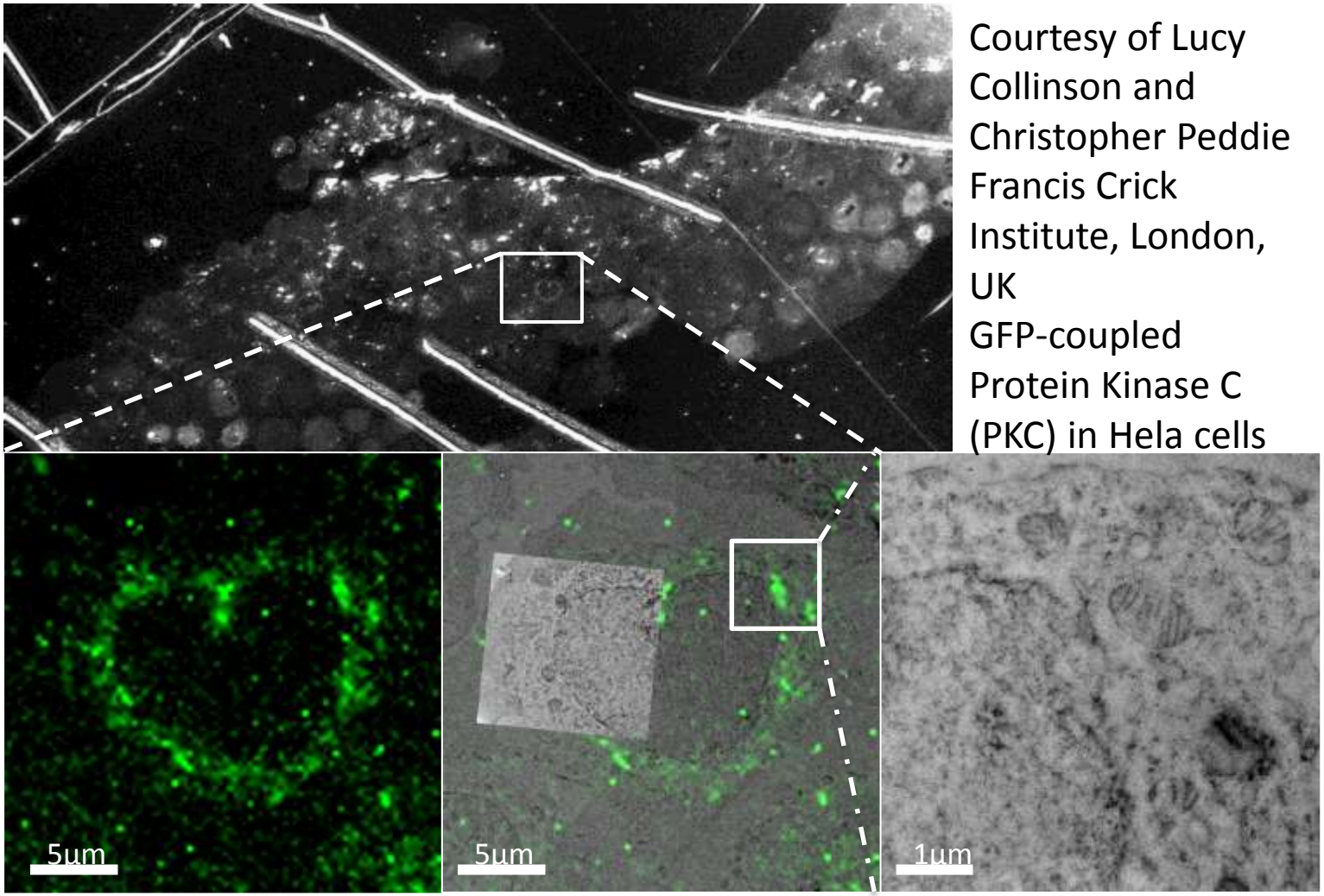




Courtesy of Nicholas Smith and Melissa Wong, Oregon Health and Science University.

Mouse intestinal tissue (Paneth cells), immuno-labeled for lysozyme

iCorr: GFP labeling



Courtesy of Lucy Collinson and Christopher Peddie
Francis Crick Institute, London, UK
GFP-coupled Protein Kinase C (PKC) in Hela cells

CorrSight & MAPS

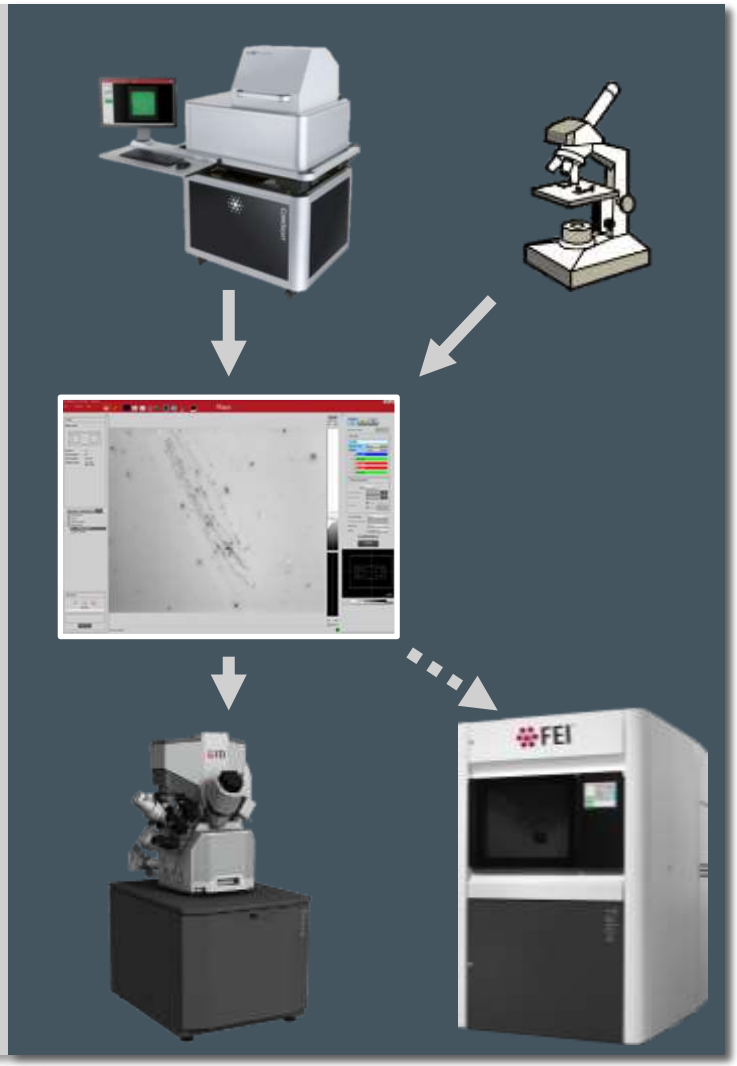
CorrSight: microscope, sample prep and navigation tool

Unique modular concept:

- From wide-field fluorescence to spinning disk confocal depending on the need
- Immobile sample stage allowing complex sample environments
- Easy exchange of sample environments for different experiments

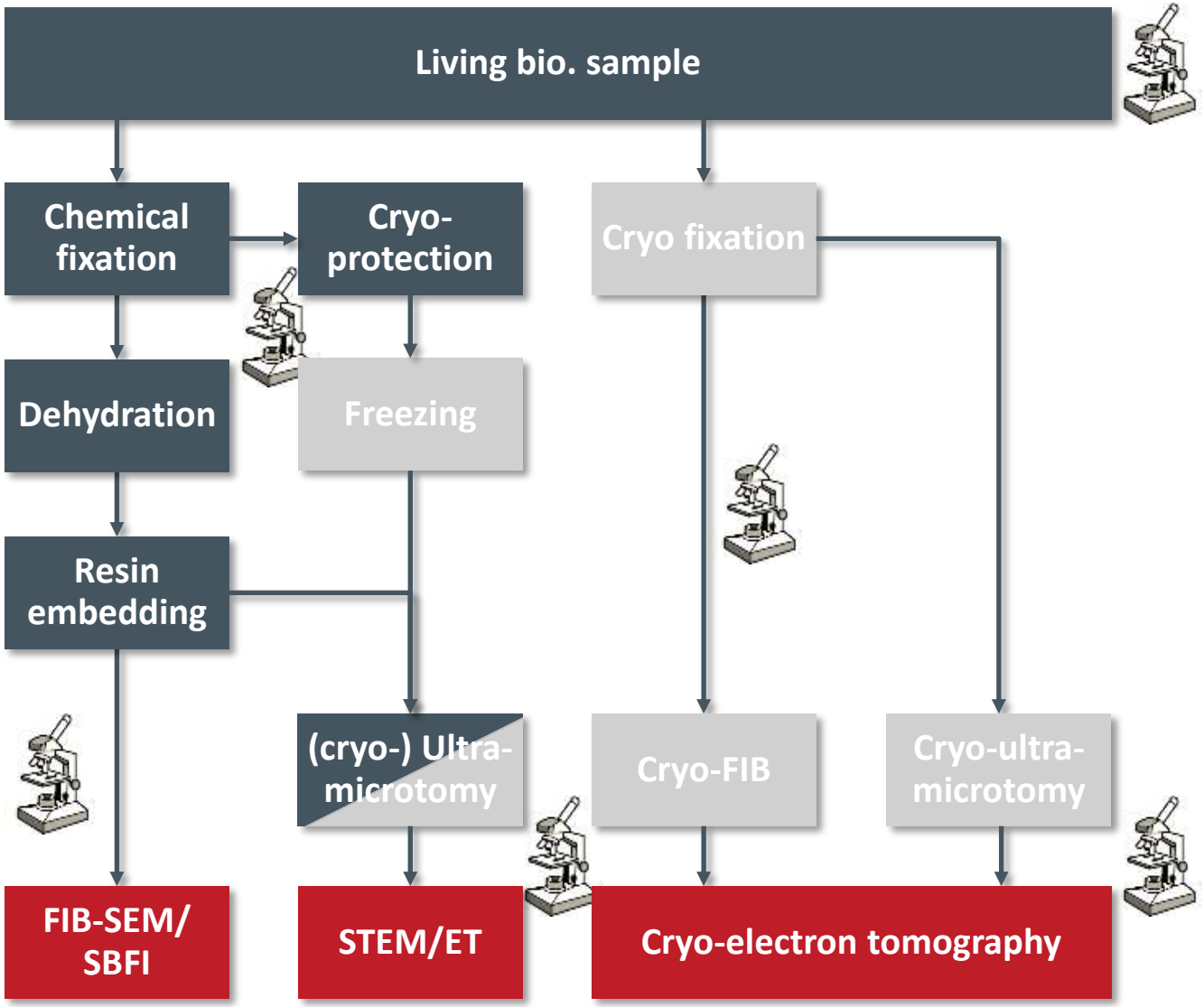
MAPS: A unified software interface for CLEM

- MAPS bridges the CorrSight and SEM/SDBs
- Makes navigation and image correlation fast and intuitive
- Provides tiling and stitching
- Open software platform can import and correlate any image
- Will be available for TEMs as well



Prescreening of EM samples

Sample preparation workflows



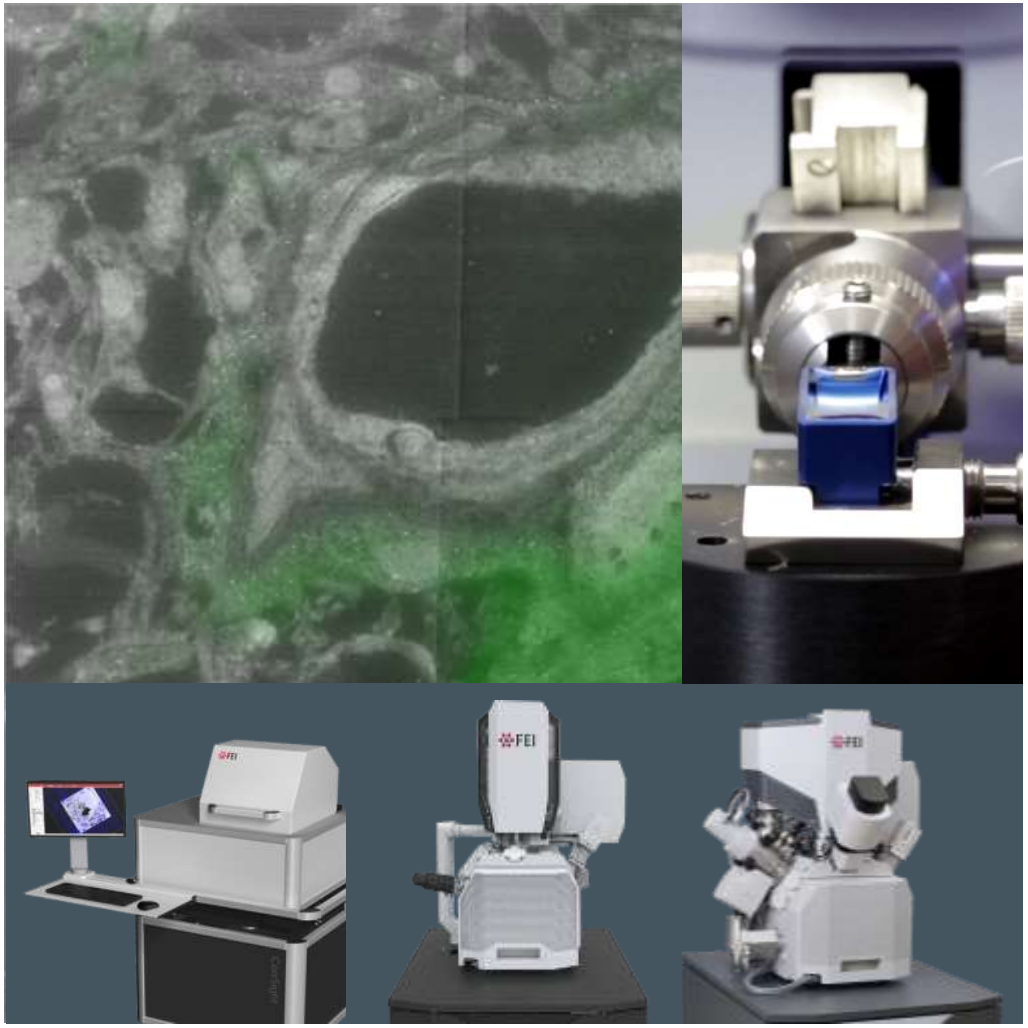
Experimental steps

Grow cells/tissue
with fluorescent label

Embedding sectioning

LM imaging to Localize ROIs
(CorrSight screen)

EM acquisition on ROIs
(SDB/SEM/VS)

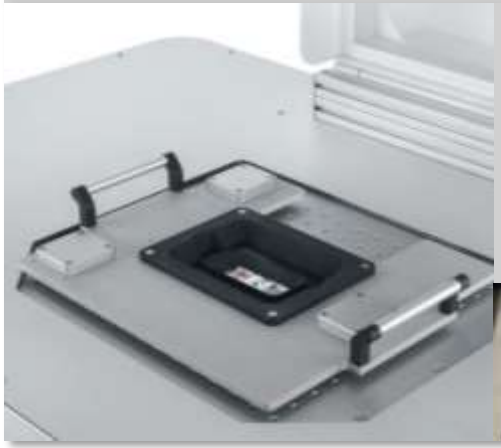


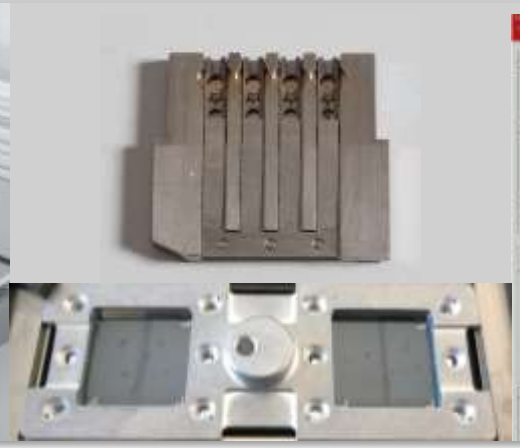
Courtesy of C. Loussert-Fonta and B. Humbel, UNIL

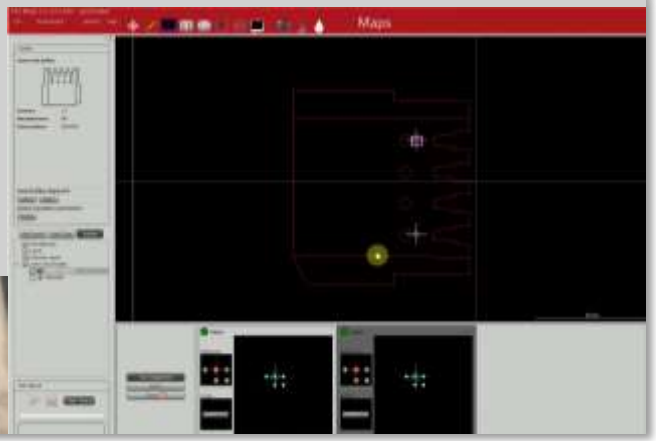
Correlative sample holders for automated fiducial-based correlation

Automated scanning of multiple samples to identify promising areas

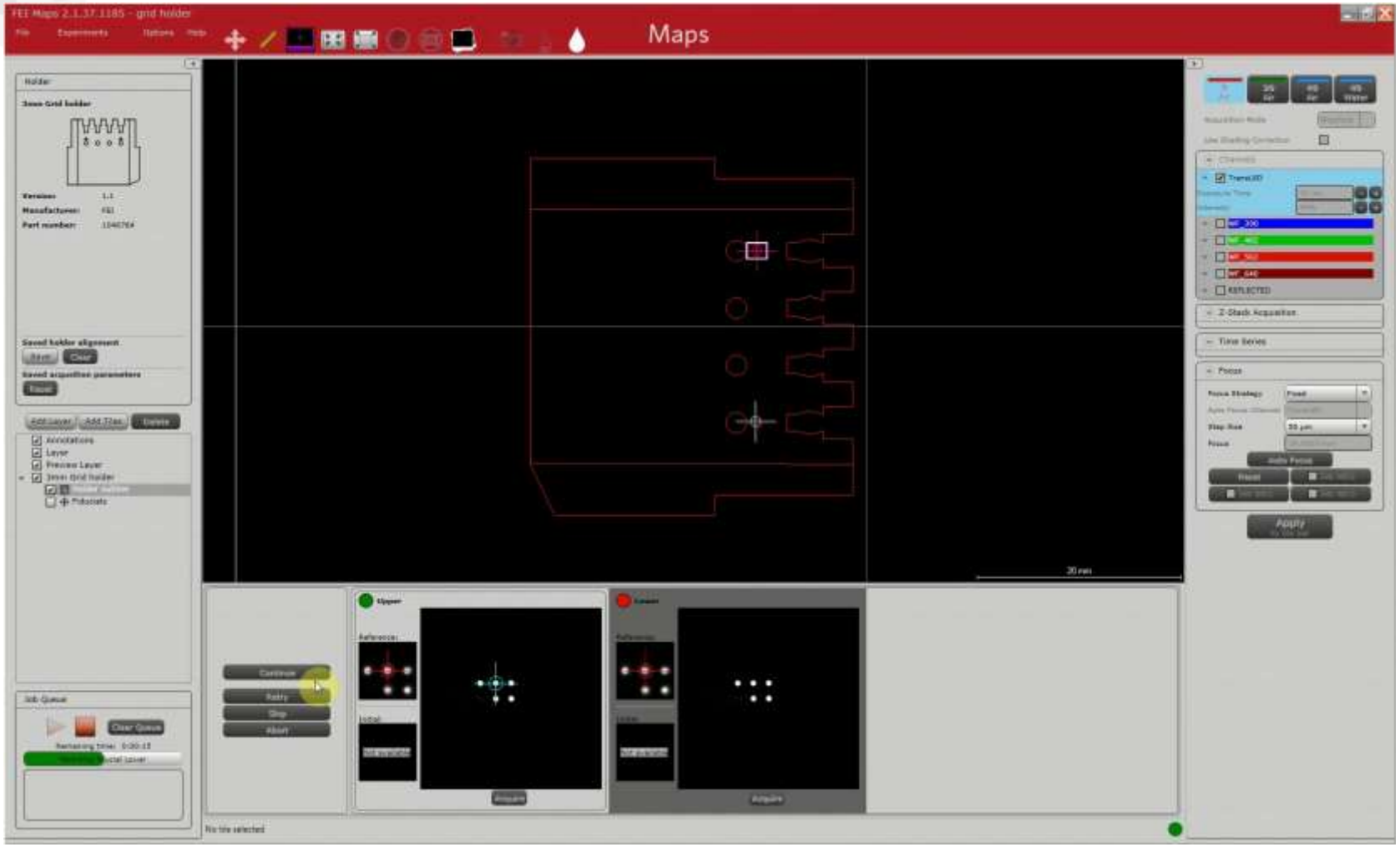
- Sample holders **directly compatible** with CorrSight and SEMs
- Available for a variety of sample formats
  - **ITO** slides
  - TEM **grids**
- Fiducials for **automated correlation**



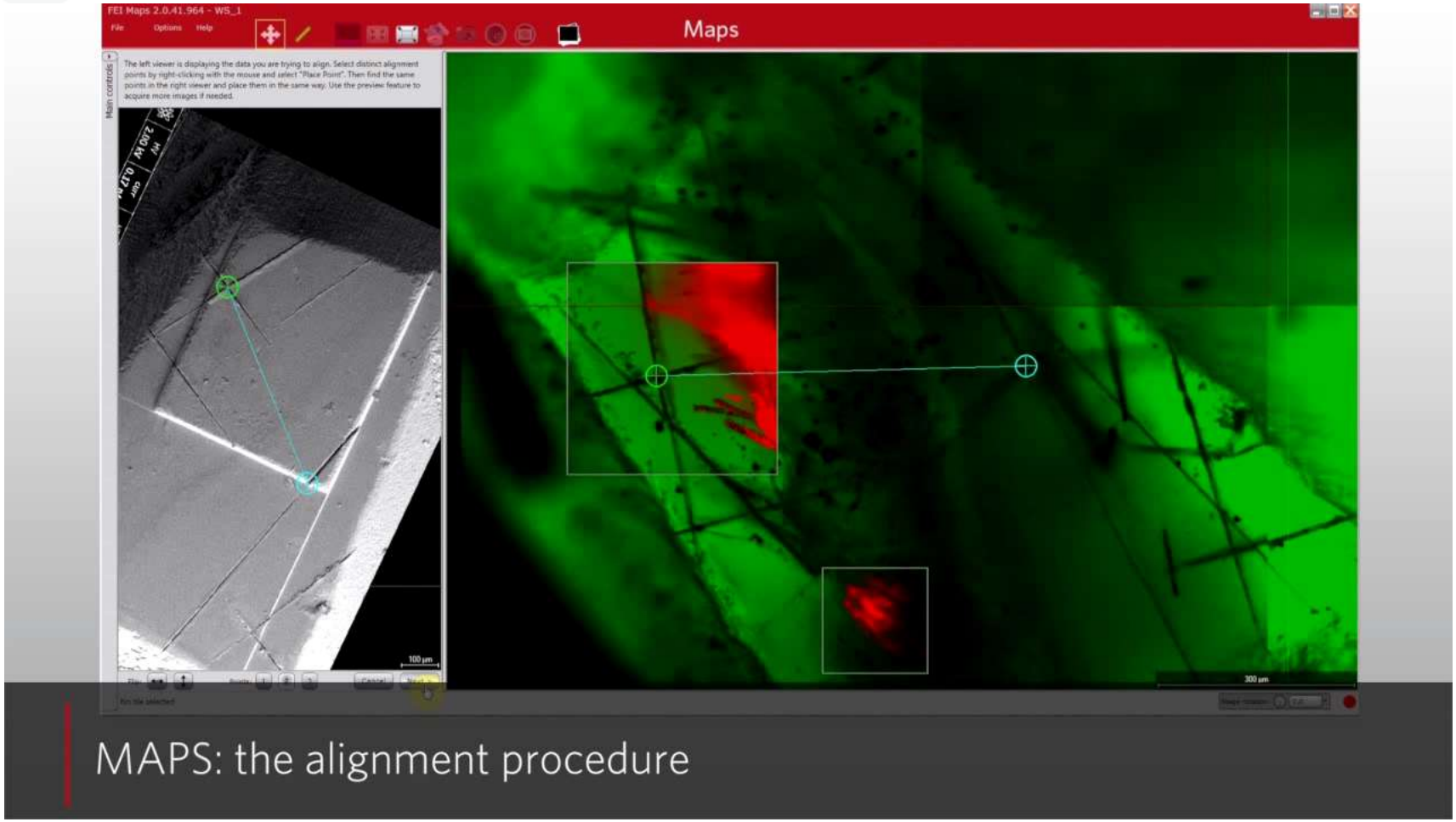




Automatic fiducial-based alignment



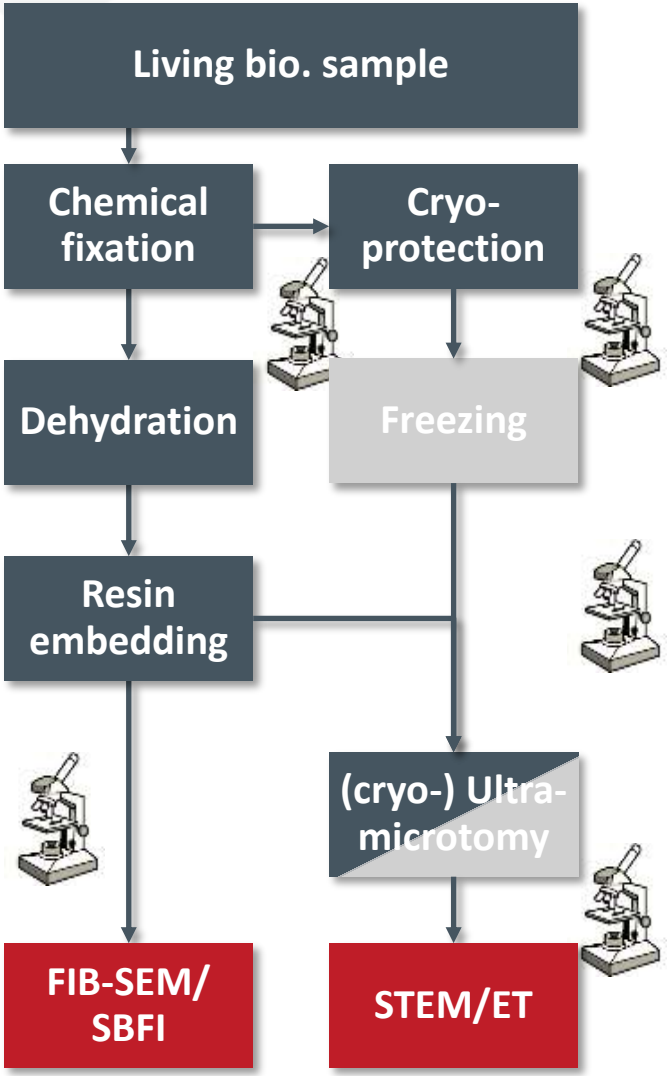
LM & EM data acquisition and correlation using MAPS



Mouse skin tissue, courtesy of ETH

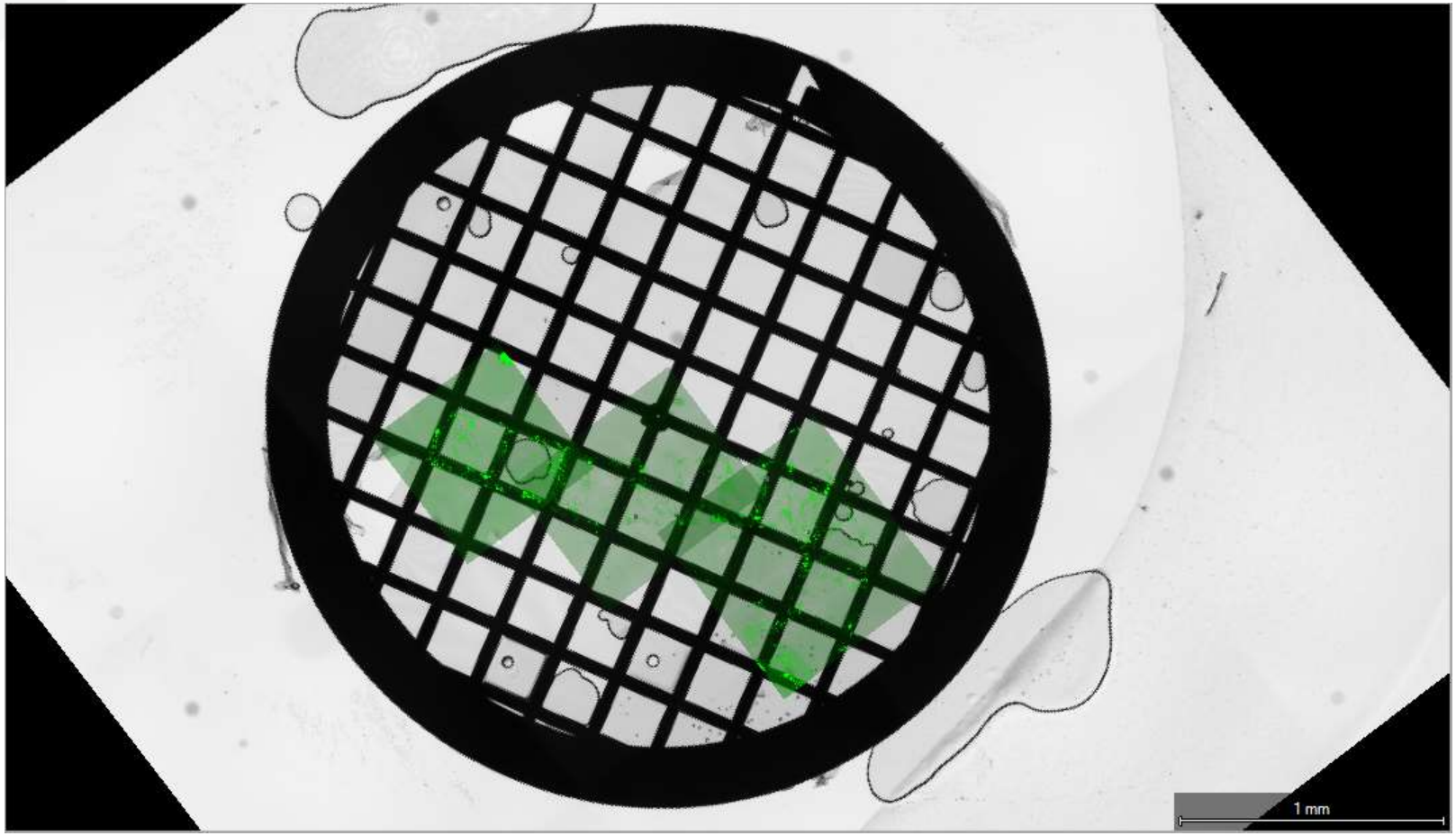
Analysis of acute brain slices by electron microscopy: A correlative light–electron microscopy workflow based on Tokuyasu cryo-sectioning.

Loussert Fonta, C., et al. J. Struct. Biol. (2014)



- Preparation of acute brain slices: 300 micron sections cut with vibrotome, then transferred to buffer containing 4% PFA and 0.5% GA
- Cutting of smaller pieces and cryo protection with sucrose and mounted on aluminium pins, plunged in liquid N_2
- Cryo sectioning, 70-100nm, taken up in methylcellulose and sucrose, warmed to RT, transferred to finder grids
- GFP immuno gold labeling
- Embedded in thin film of methylcellulose containing 0.3% uranyl acetate, air dried
- STEM imaging on Helios

STEM imaging of astrocytes in Tokuyasu-prepared brain sections



Data courtesy of Celine Loussert-Fonta & Bruno Humbel

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HEC Lausanne

From live-cell imaging to 3D ultrastructure

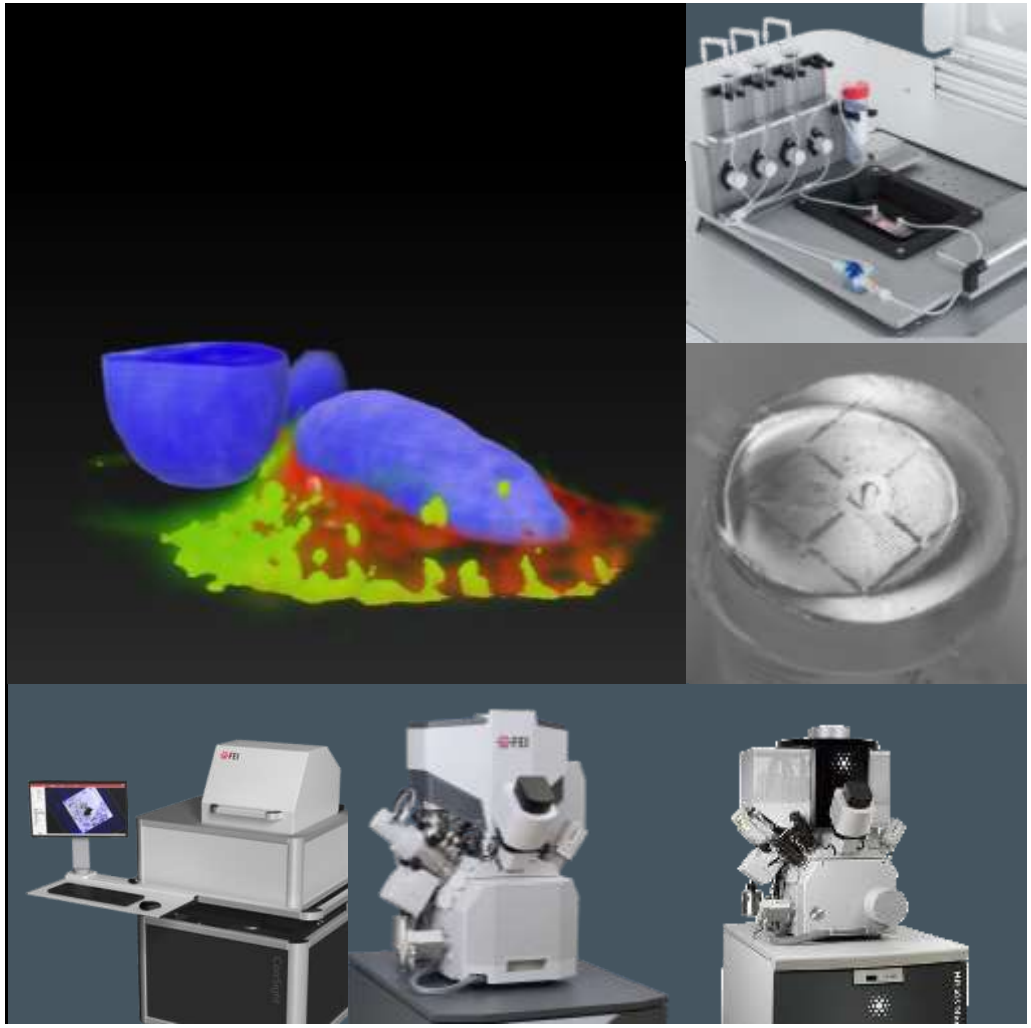
Experimental steps

Cell culture ibidi µ-slides

LM imaging to identify areas of interest; in-situ fixation, staining, embedding

LM/EM with MAPS
3D serial sections acquisition on area of interest wit SDB/SEM/VS

Reconstruction & visualization of 3D serial section dataset with AMIRA



Courtesy of S. Kwon and C. López, OHSU

CorrSight Live

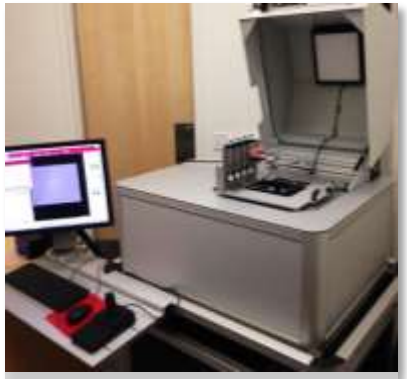
Sample environment

- Perfusion
- Heating
- CO_2 incubation

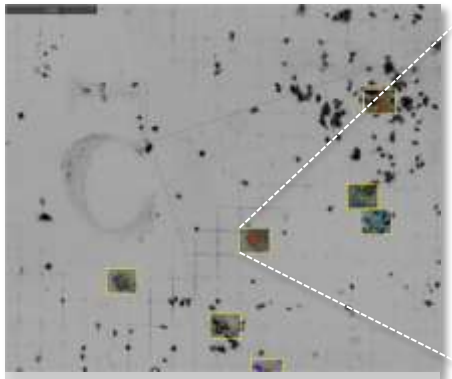
Microfluidic chamber

- Open wells allowing **easy handling** of different samples
- Closed by foil for the experiment to allow controlled **closed perfusion**
- **Optical quality bottom** (170 µm thickness)
- **Grid coordinate system** imprinted on the bottom

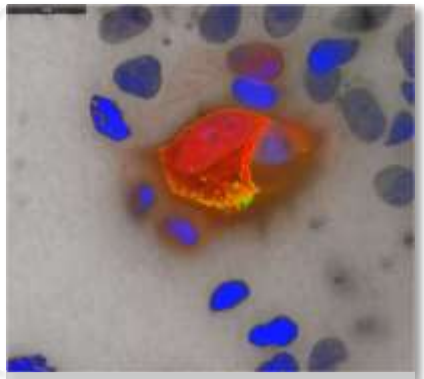
CorrSight Live: imaging and fixation of MCF-7 adherent breast cancer cells



Live cell imaging and µ-fluidics module



MAPS tiling/stitching large area overview – transmitted light and fluorescence microscopy



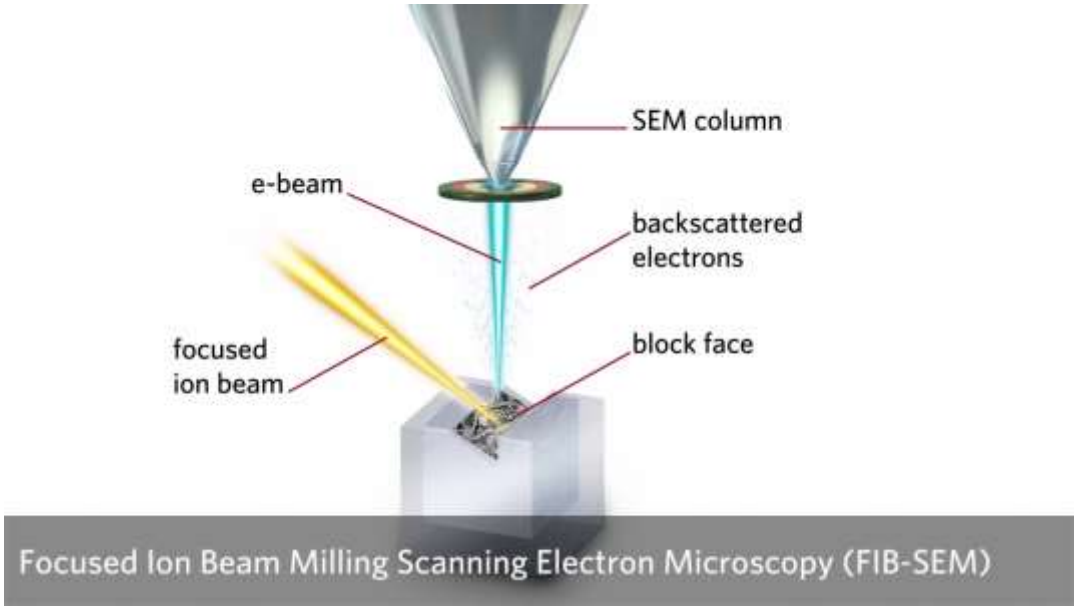
Area of interest identified by fluorescence microscopy

fixation, staining, resin-embedding directly on µFluidics module

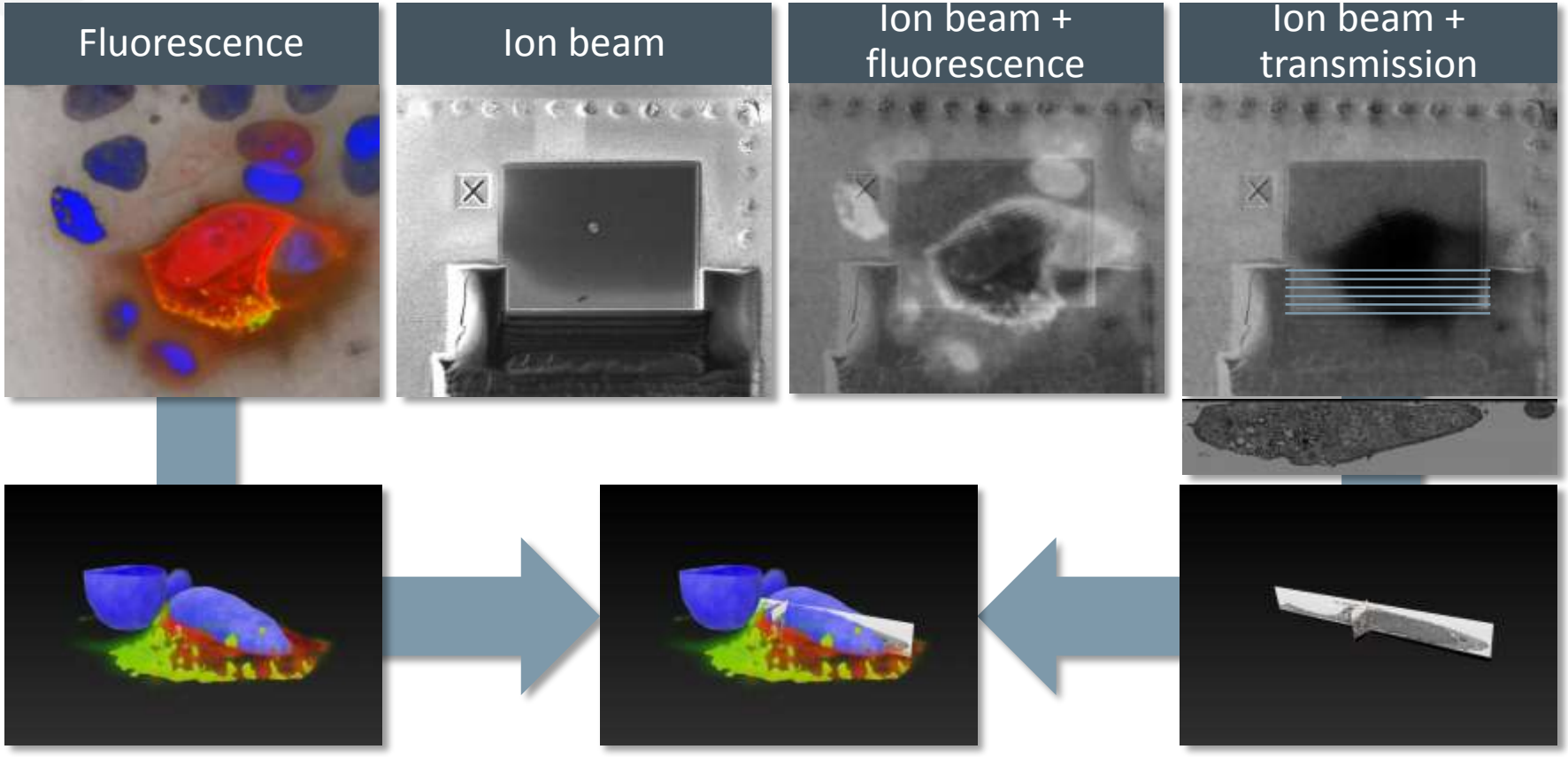
Courtesy of S. Kwon and Claudia López

3D electron microscopy: FIB-SEM/DualBeam

- Using focused ion beam for automated sectioning and imaging of the freshly cut block face
- Walk-away acquisition of volumes (200 μm^3)
- Curtaining artifacts on block face
- Best axial resolution (3nm)
- Lateral resolution limits set by image acquisition times



3D CLEM on plastic-embedded MCF-7 adherent breast cancer cells



Courtesy of S. Kwon and Claudia López, OHSU

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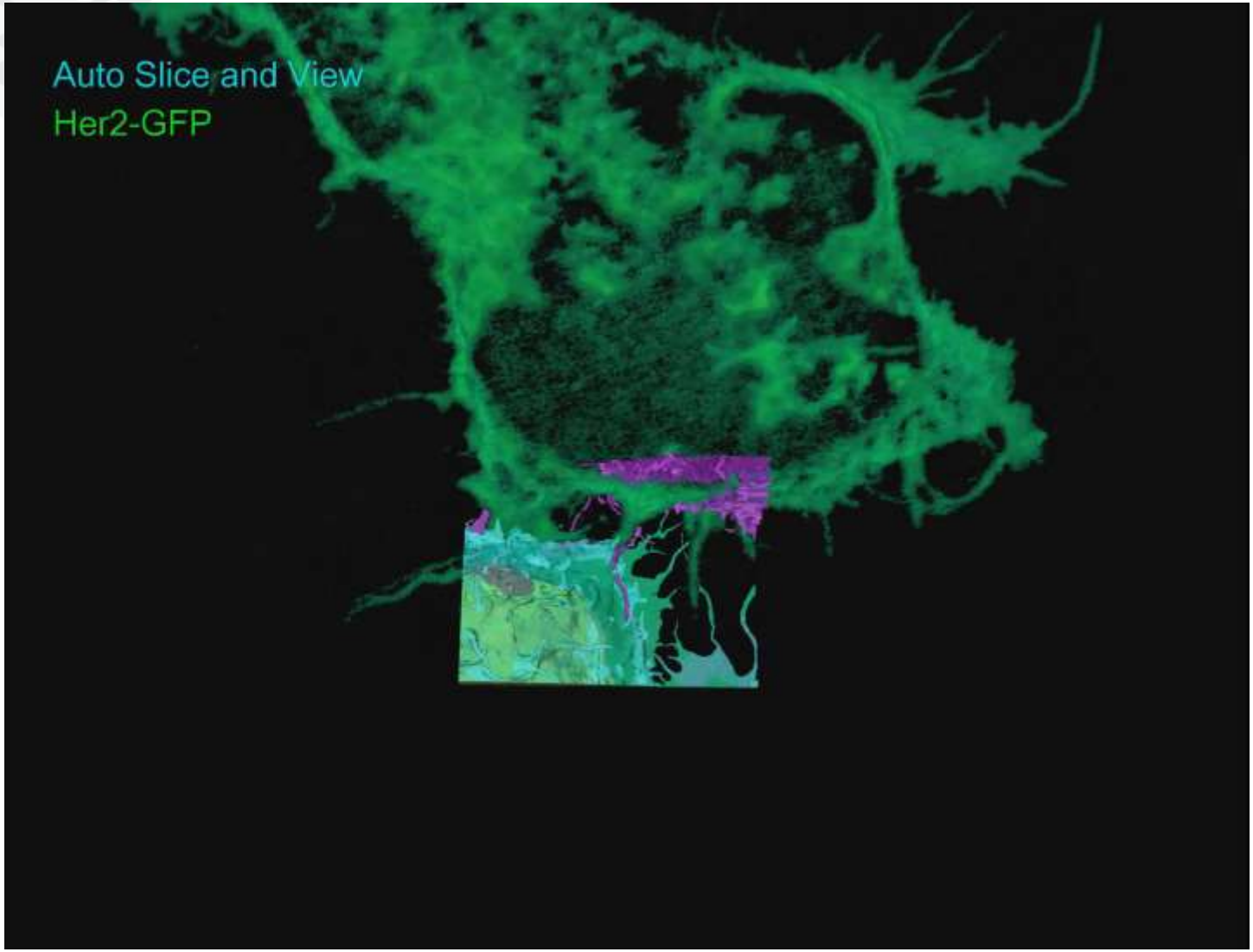
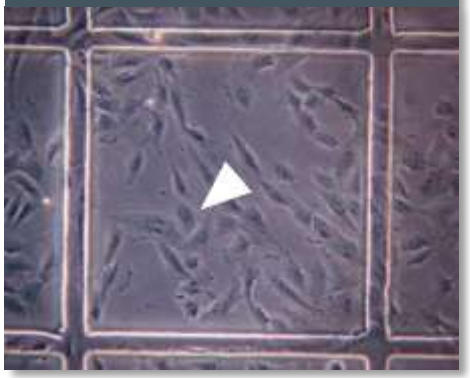
MCF-7 adherent breast cancer cells



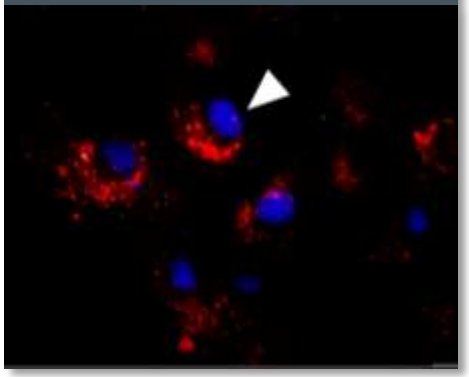
Courtesy of S. Kwon and Claudia López

Tracking lysosomes in retinal pigment epithelium cells

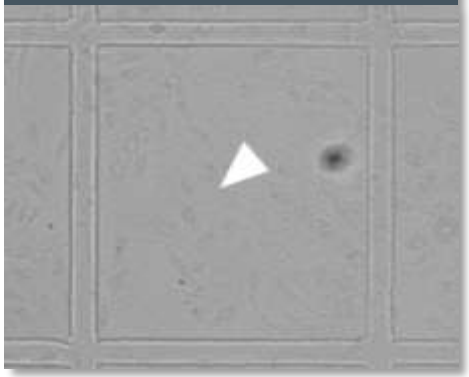
Transmission



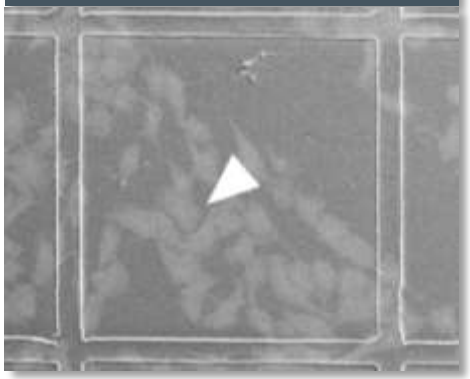
Fluorescence



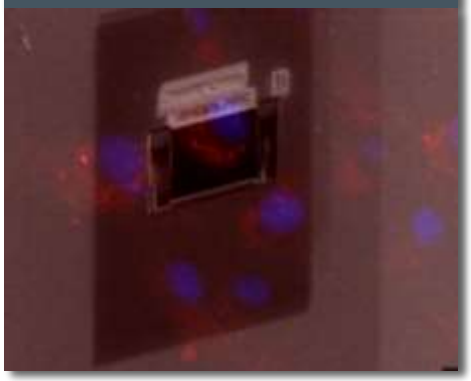
TM (embedded)



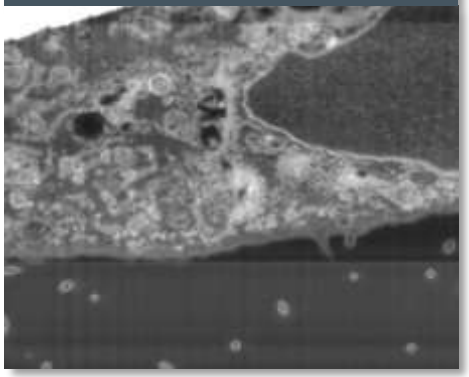
backscatter image



after Slice&View



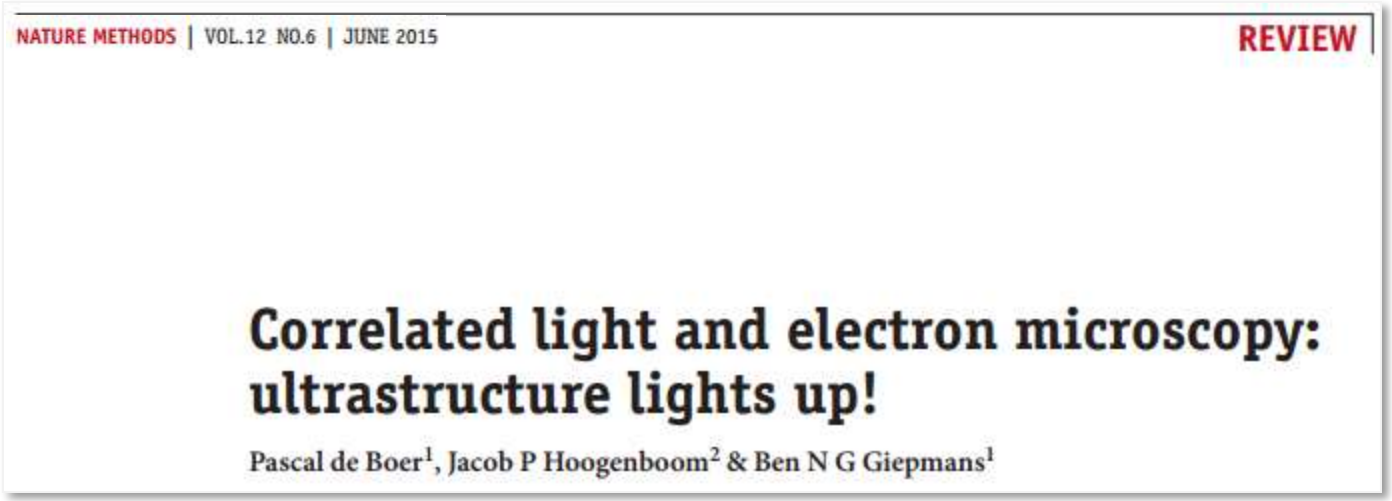
slice from S&V



Courtesy of R. Wepf

ETH

Further reading…



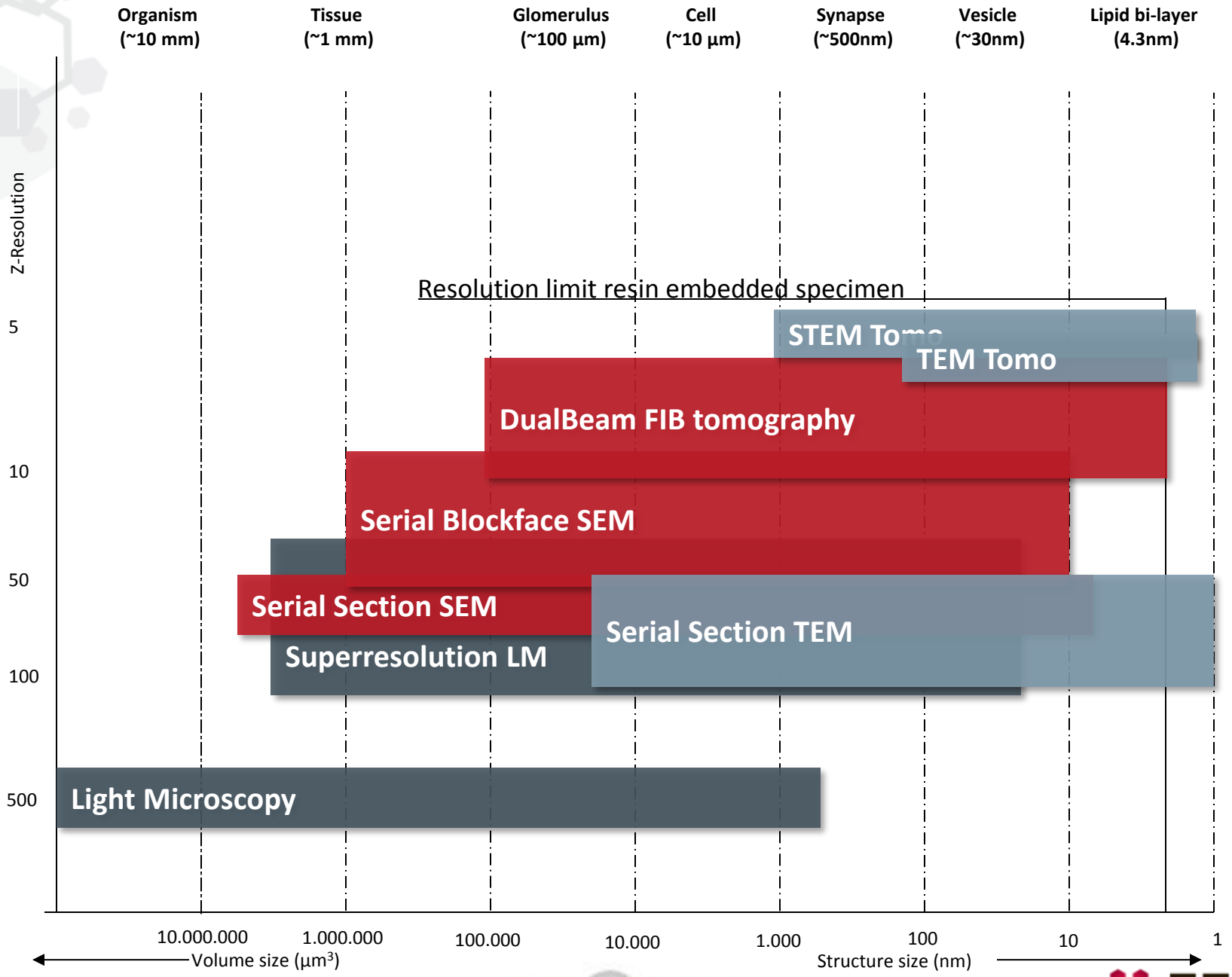
NATURE METHODS | VOL.12 NO.6 | JUNE 2015

REVIEW

Correlated light and electron microscopy: ultrastructure lights up!

Pascal de Boer[1], Jacob P Hoogenboom[2] & Ben N G Giepmans[1]

Tissue biology solutions



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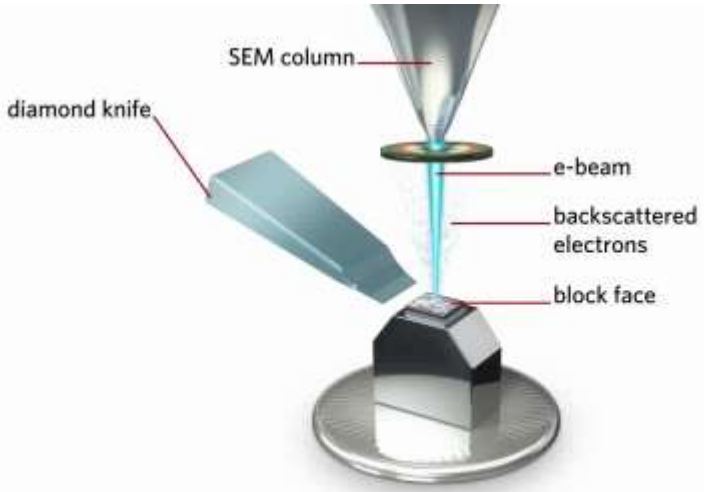
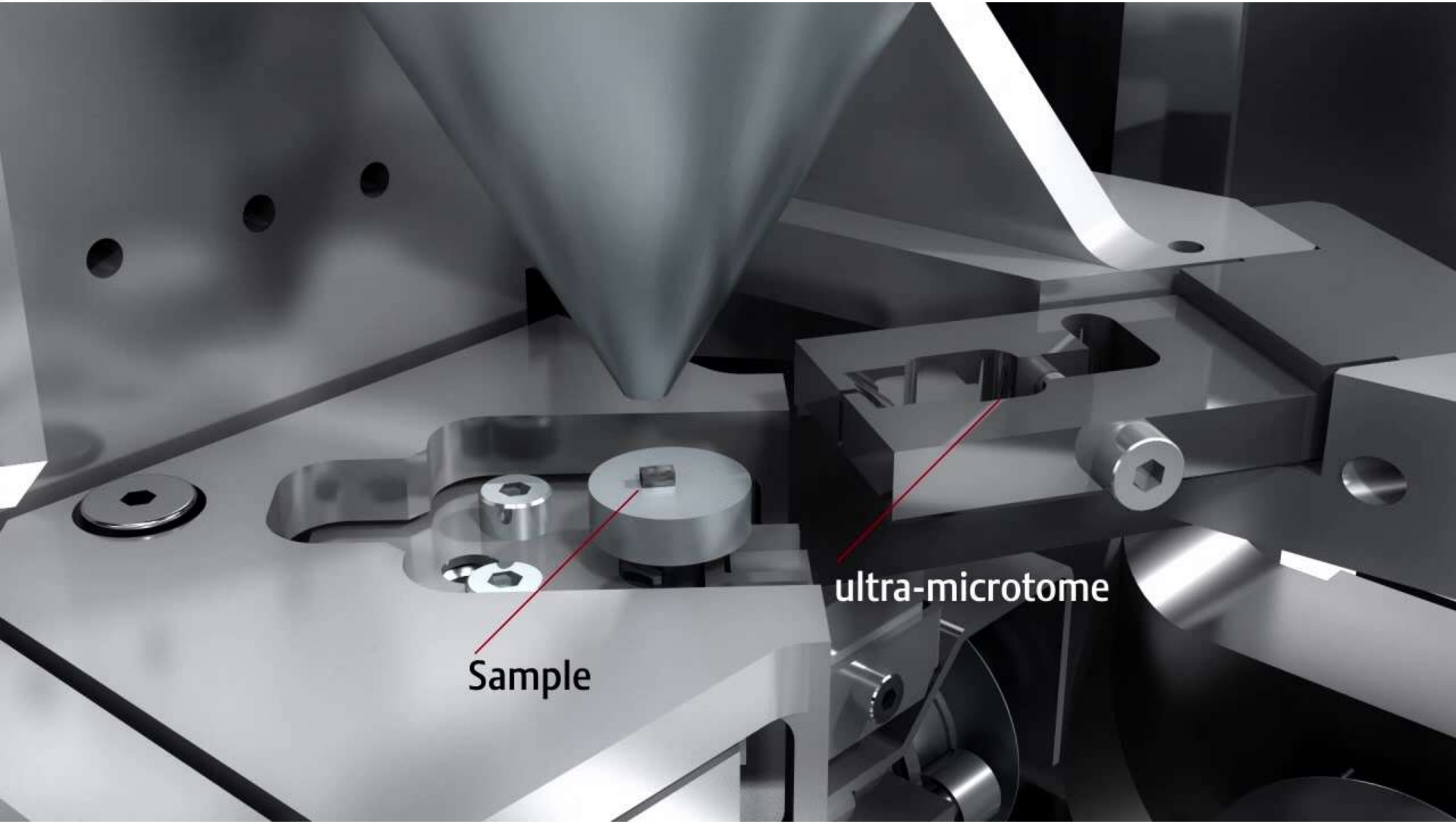
Serial Block Face Imaging

- *In-situ* ultramicrotome for automated sectioning and imaging of the freshly cut block face
- Walk-away acquisition of large volumes (100s of μm^3)
- Less knife artifacts on block face
- **But:**
  - Limited axial resolution (sectioning thickness, practical limit around 25 nm)
  - Lateral resolution limits set by image acquisition times



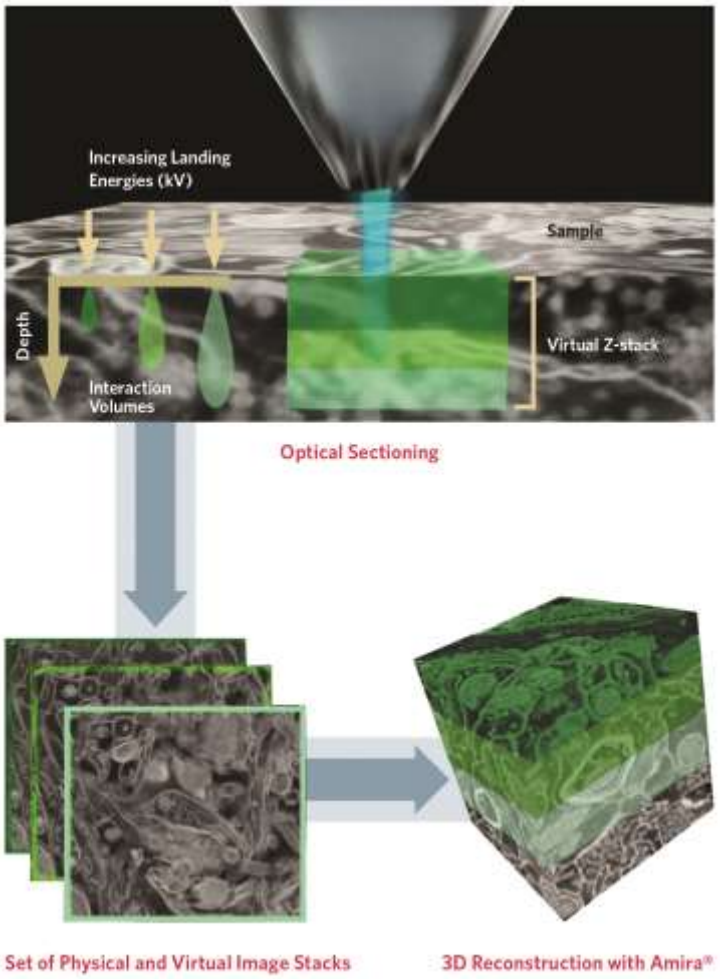
Teneo VS



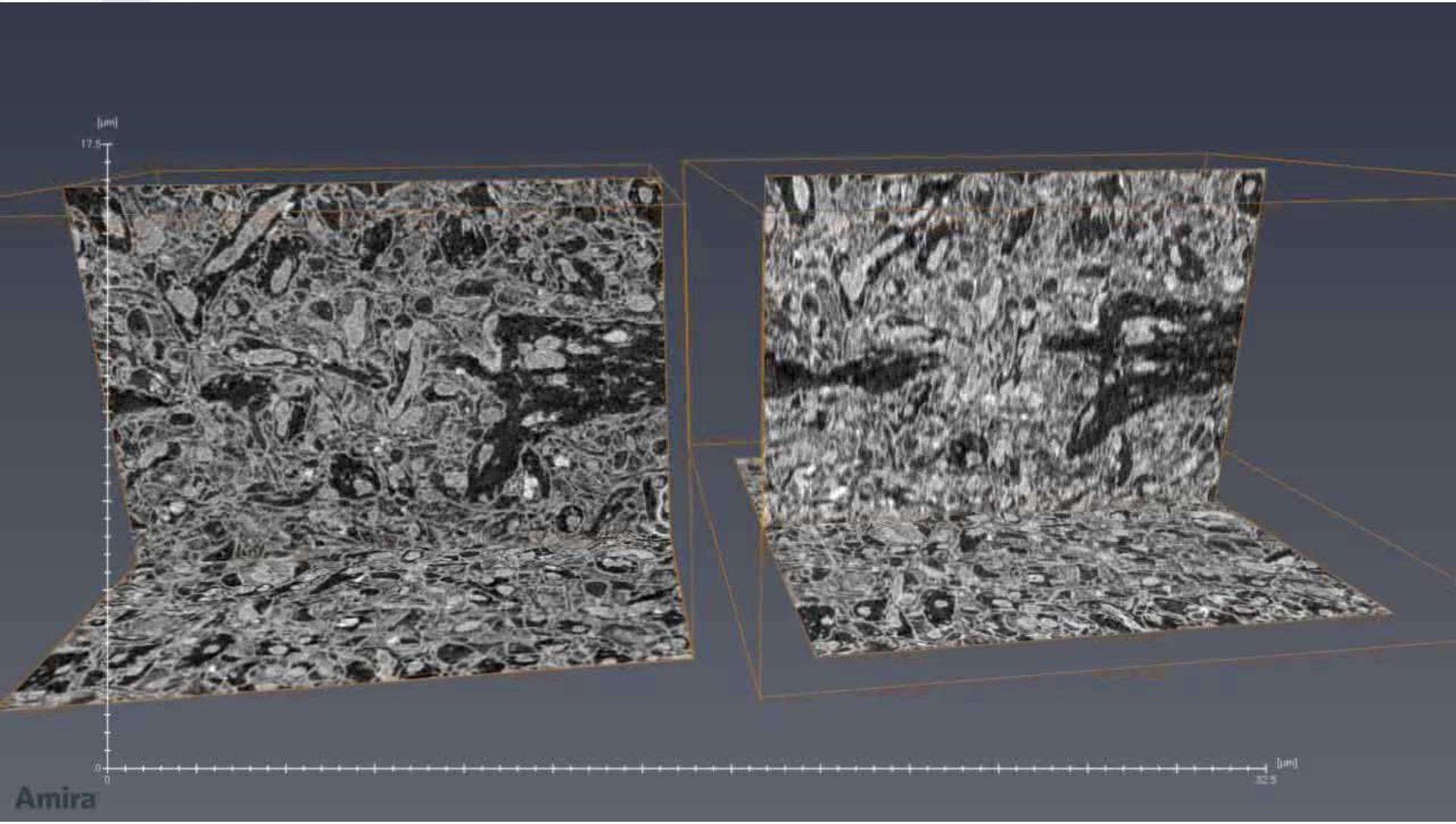
Isotropic data – Multi-energy deconvolution

- Acquire an image series with increasing energy

  $\Rightarrow$ increasing interaction volume
- Blind deconvolution to create virtual z-stack
- 70 nm depth info @ ≥ 10 nm isotropic resolution
- patented technology



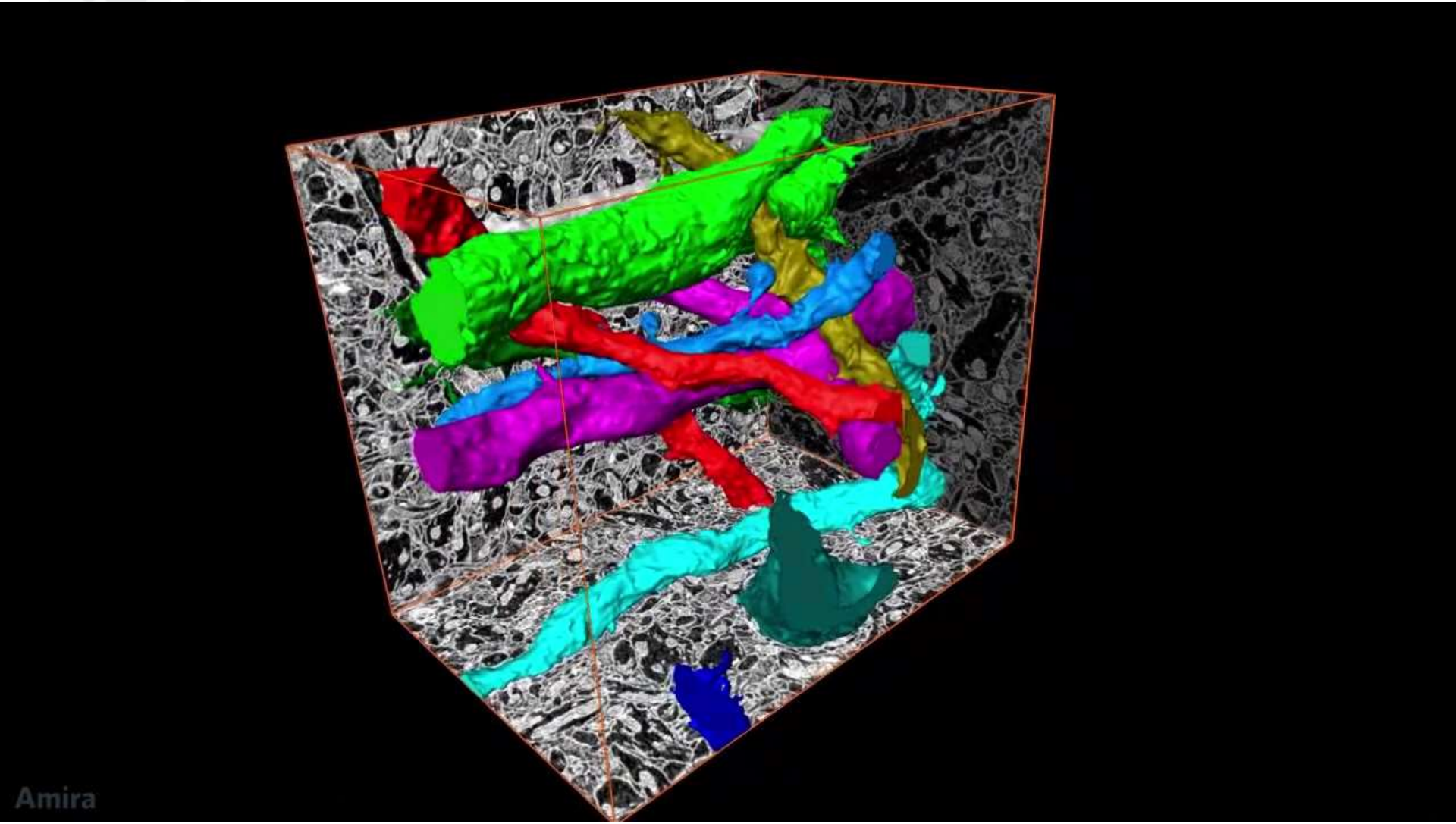
Comparison SBF-SEM vs SBF-SEM+MED-SEM



Physical slices + optical slices: z-resolution = **10nm**

Physical slices only: z-resolution = **50nm**

Volume reconstruction of mouse brain



Volume reconstruction of mouse kidney

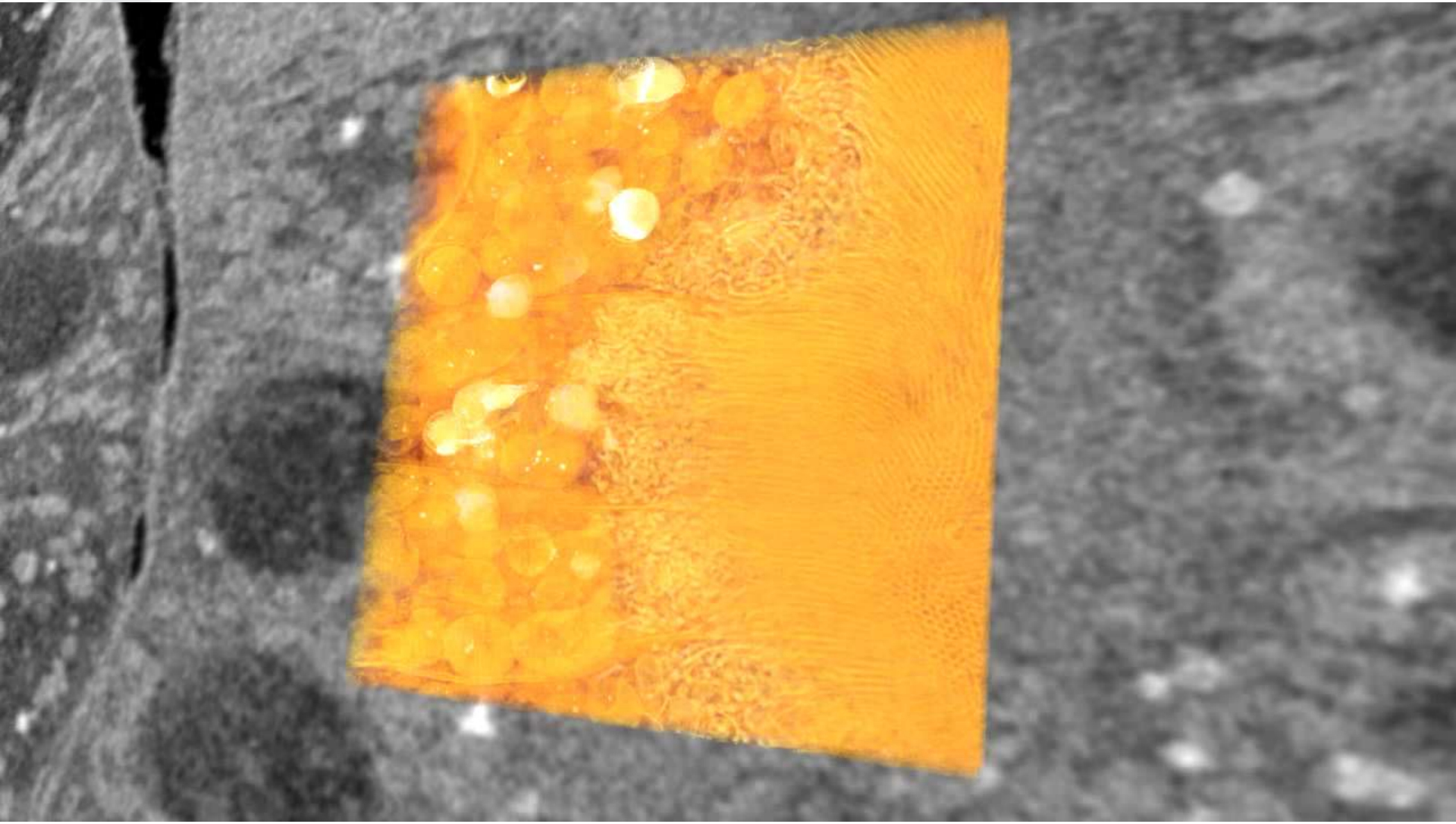
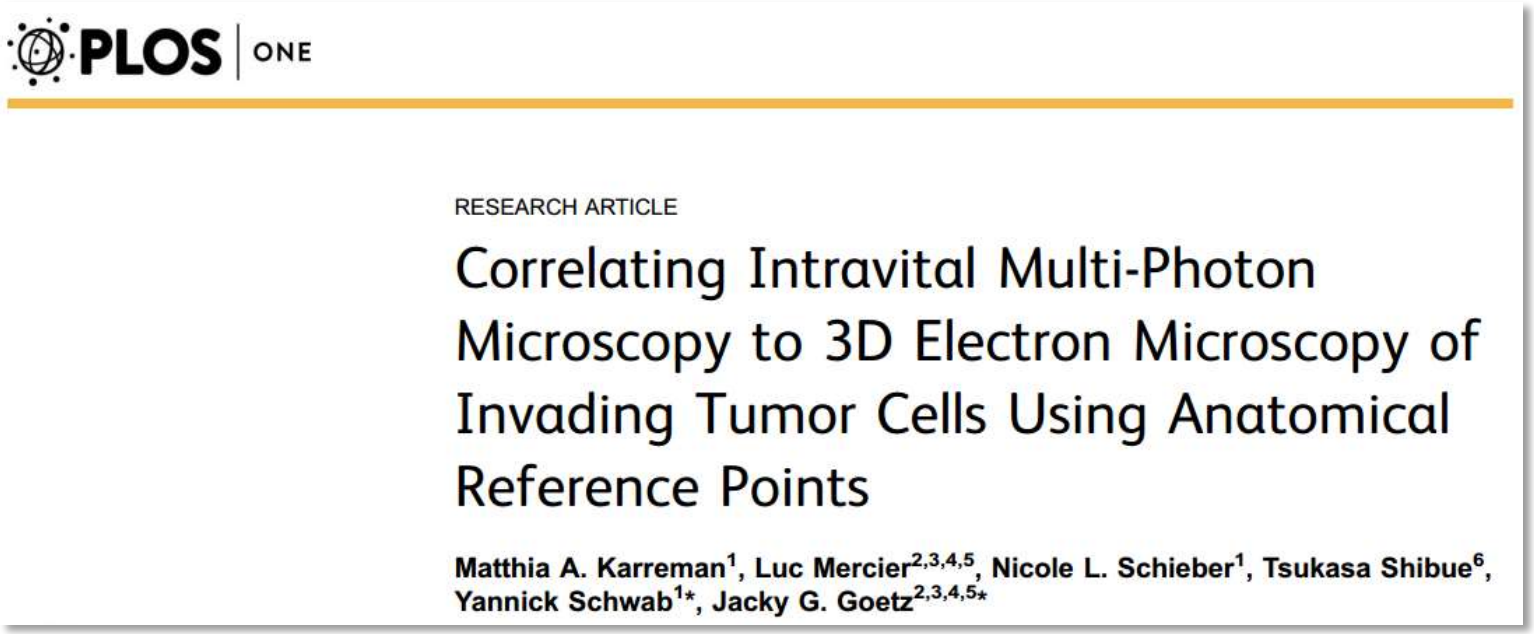


Image registration of 3D CLEM data using Amira

Intravital 2-photon to high-resolution EM

please refer to the original, open access, publication:



PLOS ONE

RESEARCH ARTICLE

Correlating Intravital Multi-Photon Microscopy to 3D Electron Microscopy of Invading Tumor Cells Using Anatomical Reference Points

Matthia A. Karreman[1], Luc Mercier[2,3,4,5], Nicole L. Schieber[1], Tsukasa Shibue[6], Yannick Schwab[1]*, Jacky G. Goetz[2,3,4,5]*

http://doi.org/10.1371/journal.pone.0114448

EMBL European Molecular Biology Laboratory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